THE MECHANISM AND SITE OF ACTION OF CLONIDINE IN THE RAT

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Jeremy Adler

Summary THE MECHANISM AND SITE OF ACTION OF CLONIDINE IN THE RAT

The inactin anaesthetized rat shows cardiovascular reflex responses. Heat rate is under sympathetic but not vagal control.

Clonidine reduces the heart rate and blood pressure in the inactin anaesthetized rat. The reduction in heart rate involves reducing sympathetic cradiac drive. The fall in blood pressure includes a reduction in peripheral resistance.

Using a newly developed "delayed" hindlimb perfusion the reduction in peripheral resistance was seen to be neurally mediated. A peripheral vasodilator action was not seen with clonidine.

Clonidine was administered by four different routes which were expected to provide access to selected areas in the brain. Intravenous, intracarotid artery, intraventricular and intravertebral artery. Administration into the ventricular system of the brain was slighty more potent in reducing arterial pressure than intravenous injection. Intracarotid and intravenous were equipotent. Intravertbral was by far the most effective, requiring 5% of the intravenous dose to cause an equivalent cardiovascular response.

Autoradiography with ^{3}H-clonidine was used to locate the injected clonidine. The new CEA Verken tritium sensitive film was used and proved able to detect very low levels of tritium. Each route of administration resulted in a different pattern of distribution.

Clonidine administered intravenously distributed evenly throughout the CNS.

Intacarotid administration selectively reached rostral areas.

Intraventricular administration had the spread limited to periventicular areas.

Intravertebral clonidine reached the medulla, pons, areas of the cerebellum and upper areas of the spinal cord.

Comparison with the different hypotensive effects led to the conclusion that the site of action was within the medulla but not in the periventricular areas.

CONTENTS

Abbreviations

Clon clonidine

GTN glyceryltrinitrate

IC intracarotid artery

ICV intraventricular

IM intramuscular

IP intraperitoneal cavity

IT intrathecal

IV intravenous

IVert intravertebral artery

Kd dissociation constant

n nano. As in ng nanogram

p pico. As in pg picogram

pA2 log (1/concentration). Concentration of an antagonist requiring doubling of the agonist concentration to restore the original response.

SC subcutaneous

SHR spontaneously hypertensive rats

T1/2 half life, time taken for decline to half initial level

u micro. As in ug micrograms or ul microlitre

5HT 5-hydroxytryptamine

6OHDA 6-hydroxydopamine

Notes

1) Mean blood pressure is obtained from systolic and diastolic:

$$\text{Mean Pressure} = \text{Diastolic} + \frac{\text{Systolic} - \text{Diastolic}}{3}$$

2) Points on graphs are given with the standard error of the mean. In its abscence the point represents only one result.

3) Only pages containing text and tables are numbered. Numbering appears at the top right corner of each page and appears as a chapter number followed by a page number. Page numbering starts anew for each chapter.

4) Tables of results appear at the end of the relevent chapter. The times noted are given with respect to the experimental manipulation concerned e.g. -5 is five minutes before. The number given at time 0 is the absolute value and those given at other times are deviations from the time 0 figure.

Drug list

Drug	Supplier
Acetylcholine hydrochloride	Sigma London
Atenolol	ICI
Atropine sulphate	Sigma London
Clonidine hydrochloride	Boehringer Ingelheim
^{3}H Clonidine hydrochloride	The Radiochemical Centre Amersham
Glyceryltrinitrate	Macarthys
Inactin (Thiobutabarbitone)	BYK
Isoprenaline (Isoproterenol)	Sigma London
Phenylephrine hydrochloride	Sigma London

INTRODUCTION

The remit of this research project was to study the site and mechanism of action of centrally acting antihypertensive drugs.

Centrally acting antihypertensive drugs have their locus of action within the CNS in contrast to diuretics, ganglion blockers, alpha adrenoceptor antagonists, vasodilators, angiotensin converting enzyme inhibitors, catecholamine depleters and Beta adrenoceptor blockers. Centrally induced hypotension is characterized by a reduction in vasoconstrictor nerve activity despite the fall in blood pressure. This condition if generated peripherally classically leads to a reflex increase in vasoconstrictor nerve tone.

Clonidine (Kobinger 1978), alpha methyldopa (Scriabine et al 1976) and certain Beta adrenoceptor blockers (Lewis et al 1975 and 1976) have central actions and find a clinical role in the treatment of hypertension. Some B blockers cross the blood brain barrier (Garvey & Ram 1975) and have central actions but as a class their action is peripheral although the hypotensive mechanism is poorly understood (Scriabine 1979). The hypotensive effect of alpha methyldopa only becomes apparent over several hours (Henning & van Zwieten 1968), however clonidine acts within minutes making it easier to manipulate experimentally. Therefore clonidine was the centrally acting antihypertensive used in this research project.

Clonidine causes a fall in blood pressure and heart rate in man and animals that is rapid in onset and persists for several hours. The active dose is small, in the low microgram/Kg range, whilst Beta blockers and methyl dopa are given in the low milligram/Kg range. Despite being a very potent pharmaceutical clonidine's clinical use is limited by side effects which are apparent at the antihypertensive dose. They are a hypertensive action at high doses, rebound hypertension when a course of treatment is precipitously ended, sedation and reduced salivary secretion.

If the pharmacological properties associated with clonidine's hypotensive action were established it would increase the possibility of designing a new agent devoid of side effects. This would have important clinical implications.

To separate the pharmacological properties from those

attributable to pharmacokinetics it is necessary to establish the site of action and then proceed with the application of electrophysiological, biochemical and pharmacological techniques to reveal the requirements for an antihypertensive action. Looking at clonidine's pharmacological properties in isolation from the hypotensive action is of use but does not necessarily demonstrate those required for hypotension. Therefore the establishment of the site of action is of prime importance in the understanding of the hypotensive effect.

This study investigated the mechanism and site of action of clonidine in the anaesthetized normotensive rat.

A REVIEW OF THE CURRENT STATUS OF CLONIDINE

Clonidine, also known as St 155, Catapres, Catapresan and DCAI was developed as an alpha adrenergic agonist for use as a nasal decongestant in the early sixties by Boehringer Ingelheim and serendipitously proved to have antihypertensive properties. An alternative story (Bowman & Rand 1980) has clonidine proposed as an additive for shaving cream, its alpha agonist action serving to constrict severed vessels reducing bleeding and also to contract the erector pili muscles making for a closer shave.

Clonidine has seen limited clinical use in blood pressure control, has served as a tool in understanding the cardiovascular system, played an important role in the subdivision of alpha adrenoceptors, aided in the recognition of presynaptic receptors, seen use in the treatment of glaucoma and shown a potentially important interaction with opiates in analgesia and withdrawal. This multiplicity of roles has led to clonidine's representation in around 2000 papers becoming a minor research area in its own right.

The Actions of Clonidine

1) Cardiovascular

Clonidine is used as an antihypertensive in man for both chronic and acute cases. Given acutely, systolic, diastolic and mean arterial pressure fall, heart rate is reduced, cardiac output drops and variable changes in peripheral resistance are seen [Kroetz et al (1969), Brest (1969), Bock et al (1969), Kho (1976)]. In the supine position no consistent alterations in peripheral resistance appear, measured standing a reduction is seen (Brest 1969). The reduced cardiac output is not spread equally among tissues, skin blood flow falls while that to muscle is unchanged (Bock et al 1969). Responses to exercise, tilting and the valsalva manoeuvre are maintained although the tilt response is reduced [Muir (1969), Dollery et al (1976)]. The cold pressor response is attenuated but this may relate to the analgesic properties of clonidine (Dollery et al 1976). Orthostatic hypotension and exercise syncope are not seen with clonidine. A pressor response is seen after rapid IV dosing (Bock et al 1969), in patients already receiving hypotensive therapy (Kroetz

et al 1969) and in patients given large doses (Wing et al 1977), (Saarima 1976). Given rapidly IV a high plasma level is briefly attained and a direct peripheral action of clonidine causes vasoconstriction. In patients already on hypotensive therapy clonidine may be unable to reduce blood pressure further and a sustained rise may appear in arterial pressure reflecting a direct action on the vasculature. When high doses are administered a peripheral vasoconstrictor effect is superimposed on central hypotensive action resulting in an increase in blood pressure. A physician when faced with an inappropriate response may increase the dose, further compounding the problem. Longterm therapy leads to a restoration of cardiac output and a fall in peripheral resistance (Reubi et al 1969).

On the cessation of clonidine therapy rebound hypertension and withdrawal symptons may occur. Geyskes et al (1979) and Hunyor et al (1973) reported tachycardia, hypertension, headache, nervousness, palpitations and nausea. An increase in noradrenaline excretion also occurred. Withdrawal is not reported in all patients (Conolly et al 1969). The distinction between rebound hypertension and a return to pretreatment blood pressure and heart rate is unclear but may be assumed to occur when the levels on withdrawl exceed those prior to treatment. The non-cardiovascular manifestations are similar to those encountered with opiates.

Discussion of the mechanisms involved appears in the sections on the site of action and pharmacology of clonidine, in this chapter.

2) Gastric Secretion

Gastric acid secretion is reduced in man (Kaess & Von Mikulitz-Radecki 1971) by clonidine but under experimental conditions alterations in either direction are possible.

In anaesthetized rats clonidine 30-1000ug/Kg IV (Walz & van Zwieten 1970), 625-5000ug/Kg IP (Cheng et al 1981), 1000ug/Kg IV (Jennewein 1977) and 500ug/Kg IV (Karppanen & Westermann 1973) increased gastric acid secretion. This action is shared by histamine and both are antagonized by similar doses of histamine H2 antagonists [Karppanen & Westermann (1973), Jennewein (1973), Parsons (1978), Cheng et al (1981)] intimating that histamine H2 receptors

are involved. Phentolamine leaves the stimulant action of clonidine unaltered showing that adrenoceptors are not involved (Cheng et al 1981). Atropinisation does not reduce clonidine-induced acid secretion indicating that this action is not vagally mediated.

In unanaesthetized dogs and rats clonidine 1000ug/Kg (Walz & van Zwieten 1970), 3-1000ug/Kg (Jennewein 1977), 30ug/Kg SC (Pascaud & Roger 1976), reduced gastric acid secretion. Clonidine injected into the lateral ventricles is more potent than IV administration suggesting a central site of action . This is also implicated by clonidine's ability to reduce insulin and 2-deoxyglucose induced acid secretion, which are centrally mediated whilst the effects of the peripherally acting agents histamine, carbachol and bethanechol are unchanged or enhanced.

Clonidine reduces acid production resulting from vagal stimulation and in this role is itself antagonized by phentolamine revealing the presence of adrenoceptors, probably presynaptic. The importance of this peripheral action is hard to assess since the experimenters did not use a range of doses and stimulation frequencies.

The differences found in anaesthetized and unanaesthetized animals become explicable when it is appreciated that anaesthesia greatly reduces basal acid production by reducing vagal tone and that increased acid secretion only occurs at high doses of clonidine. Jennewein (1977) found acid production reduced in a dose related manner 3-100ug/Kg and that further increases up to 1000 ug/Kg were no more effective, Pascaud & Roger (1976) report an ED50 of 20 ug/Kg. In anaesthetized animals low doses are inactive as there is no vagal tone to reduce but as the dose rises an increase mediated by H2 receptors is seen. In unanaesthetized animals clonidine acts centrally to reduce acid secretion, as the dose rises a peripheral action on the vagus further decreasing secretion would be anticipated. However Boissier et al (1970) could not demonstrate this effect with a dose of 100ug/Kg, a dose found to reduce acid production maximally. Therefore a vagal presynaptic action probably has no role in unanaesthetized animals. A stimulant action has not been reported in unanaesthetized animals probably because sufficiently high doses have not been employed.

Clinically clonidine has not been used to treat gastric

ulceration and investigation of this area has received little attention. Some anecdotal refences were made at the 1969 symposium "Catapres in Hypertension" which suggested that clonidine was ineffective clinically (p213-214).

In conclusion clonidine acts centrally to reduce gastric acid secretion and at high doses stimulation is apparent.

3) Salivary Secretion

A dry mouth is reported by 44% of patients receiving clonidine (Houston 1981). Bock et al (1969) found salivation reduced maximally, by 80%, 50 mins after 150 ug clonidine IV in 8 subjects. In addition to the reduction in flow the sodium concentration fell slightly and that of potassium nearly doubled. The increase in potassium was progressive over the 100 mins after clonidine whilst the reduction in flow was maximal at 50 mins recovering thereafter. Dollery et al (1976) gave 300 ug orally to five subjects, salivary flow was reduced maximally at 1 and 2 hrs after administration and progressively recovered over the next 6 hours though to only 1/3 of the original level. The reduction in flow correlated extremely well with plasma concentration. Parotid pain is reported by a small proportion of patients taking clonidine, generally at the start of a meal, and may be related to the reduction in saliva production and the increase in viscosity.

Davis and Maury (1978) found clonidine to reduce the adrenaline mediated efflux of potassium from isolated rat parotid cells. Clonidine alone was inactive but in receptor binding studies antagonized dihydroergotamine. This antagonism was found with a number of imidazoles. In vivo clonidine increases potassium levels in saliva (Bock et al 1969) suggesting that a direct action on salivary glands is not involved clinically.

Patients report that dryness of the mouth is worst over the first few days of treatment and often subsides completely over 2-3 weeks (Kellett and Hamilton 1969).

Rand et al (1969) in the dog found that peripherally induced increases in salivation, by pilocarpine, were unaltered by clonidine but that increases as part of conditioned reflexes were reduced, implicating a central site of action.

Sweet et al (1977) similarly found that clonidine reduced

conditioned salivation and that St 91, an alpha agonist unable to penetrate readily the blood brain barrier, had no effect. In consequence the reduction in salivation appears to involve a central action.

4) Sedation

Sedation is the most commonly reported side effect of clonidine, occurring in 50% of patients (Houston 1981). Dollery et al (1976) with a 300 ug/Kg oral dose found peak sedation after 2 hours after a slow onset, leading to a steady recovery over the next 6 hours, but not to the predose level. Elkeles et al (1969) found that the reported level of sedation was greatest during periods of inactivity and with continual treatment tolerance appeared over 4 weeks. Kellet & Hamilton (1969) with 12 patients reporting sedation as a side effect, out of 26, also found tolerance appearing over several weeks. With one exception the affected group found no trouble concentrating during the day but fell asleep on relaxing. Hoobler & Sagastume (1969) in a longterm study found transient drowsiness 2-3 hours after dosing, tolerance appeared but an increase in the dose led to a brief reappearance.

Sedation is unlikely to be the cause of the hypotension seen with clonidine, although the time courses correlate well. Sedation is not present in all patients, tolerance to sedation is more marked than to hypotension and sedation is seen at doses in excess of the minimum hypotensive concentration.

Sedation can be demonstrated in young chicks with 20 ug/Kg IM clonidine but lasts only a few minutes (Zaimis 1969). This sedation is not reduced by phentolamine which is effective in reversing sedation produced by adrenaline, suggesting different sites of action.

Delbarre & Schmitt (1973) in chicks and mice found that of a range of adrenoceptor antagonists employed only yohimbine reduced sleeping time. Yohimbine is an alpha adrenoceptor antagonist but other alpha antagonists were ineffective or prolonged sleeping time. Phentolamine was not used in this study.

Cavero & Roach (1977) found phentolamine but not prazosin, an alpha 1 adrenoceptor antagonist, effective in reducing clonidine sleeping times in chickens.

Timmermanns et al (1981) measuring sleeping times in mice found rauwolscine and yohimbine to be effective antagonists of clonidine but reported no action for corynanthine.

In the cat clonidine suppresses paradoxical sleep (REM sleep) which is sensitive to alpha 2 but not alpha 1 adrenoceptor antagonists. In the rat yohimbine and phentolamine were ineffective and failed to alter the clonidine sleeping time (Makela & Atkonen 1980). However the dose of clonidine was high, 100 ug/Kg, and effects of yohimbine were reported between four and eight hours after treatment.

The work with antagonists suggests that the receptor involved has the characteristics of an alpha 2 adrenoceptor. Central hypotensive actions are thought to be mediated by the same receptor but Clough et al (1978) were able to alter the ratio of sedative/hypotensive action in a series of clonidine like compounds, suggesting that the two actions do not share a common receptor.

5) Analgesia/Opiate Withdrawal

Clonidine shows analgesic properties in a variety of tests on rats and mice. Paalzow (1974) using rats found that clonidine caused a dose dependent increase in the vocalization threshold and vocalization after discharge in response to electrical stimulation of the tail. No change in the motor response was noted except with 10mg/Kg whilst 79 ug/Kg of clonidine produced the analgesic effect. Mice required higher doses 2.5-10 mg/Kg. Fielding et al (1979) in a battery of tests found reactions to doses as low as 30 ug/Kg SC, phenylquinone induced abdominal constriction in the mouse, but required 2.5 mg/Kg SC for a rat tail withdrawal. In man studies on analgesia do not appear to have been undertaken, however Dollery et al (1975) using a cold pressor test found that 2 out of 5 subjects thought that the painful stimulus was reduced after clonidine and in all subjects the pressor response was reduced.

Clonidine is more potent than morphine (Fielding et al 1979) (Spaulding et al 1979a & b). The two drugs act synergistically, the combined effect is more than additive (Spaulding et al 1979a). However clonidine analgesia is distinct from opiate induced. It is insensitive to naloxone (Fielding et al 1979) (Spaulding et al 1979a), and receptor binding studies (Golembiowska-Nitikin et al

1980) do not show an action of opiates at clonidine binding sites in the cortex. Clonidine analgesia is sensitive to yohimbine but not prazosin showing that alpha 2 adrenoceptors are involved (Hoefke & Jennewein 1981). Phenoxybenzamine failed to block the tail flick induced by heat in the mouse, showing the non involvement of alpha 1 adrenoceptors (Fielding et al 1979).

Tolerance develops to clonidine analgesia (Paalzow 1978) but cross tolerance to morphine is not seen (Spaulding et al 1979a). Paalzow (1978) does not support this contention. However this is not the only possible interpretation of the data presented. The absence of cross tolerance has clinical implications with clonidine 5 ug/Kg being used to treat opiate withdrawal in man (Gold et al 1978). Conversely morphine suppresses the cardiovascular manifestations of clonidine withdrawal in the SHR, although the withdrawal-induced tachycardia was not abated (Thoolen et al 1981). This action of morphine is naloxone sensitive. Naloxone pretreatment is reported to reverse the hypotensive response to clonidine in the SHR (Farsang & Kunos 1979) but in man Watkins et al (1980) found no interaction. This may reflect the different doses of naloxone used, 0.2 mg/Kg IP in the SHR study and 0.8 mg IV in the human study. There is much evidence for a role of opiates within the cardiovascular system: in spinal shock (Holaday & Faden 1980), with inhalation anaesthetics (Arndt & Freye 1979), endotoxin shock (Holaday & Faden 1978), vagal bradycardia (Laubie et al 1979), in central control (Feldberg & Wei 1977) (Wallenstein 1979), raised blood pressure is one of the manifestations of opiate withdrawl. Blood pressure in experimentally induced hypertension develops alongside an increase in pain threshold (Zamir and Segal 1979). This appears at odds with the observation that SHRs become progressively more irritable but suggests that the relationship between the cardiovascular system and analgesia is worth investigating

The analgesic action of clonidine is thought to be mediated by both spinal and supraspinal sites (Spaulding 1979). Aghajanian (1978) recording from locus coeruleus neurones found both clonidine and morphine to reduce the number of action potentials recorded, piperoxan (alpha 2 adrenceptor antagonist) antagonized clonidine and naloxone antagonized morphine but the antagonists were specific to each agonist. Further clonidine reduced the enhanced rate of

discharge seen in morphine dependent rats given naloxone. This indicates that a common receptor does not exist in the locus coeruleus (LC) and suggests the LC as a possible central site of clonidine analgesia. The failure of either yohimbine to effect morphine analgesia or naloxone to alter clonidine analgesia implies that the separate sites of action are not sequentially located in an analgesic neuronal system or that if this is the case the rostral site is quiescent in the absence of clonidine or morphine.

Overall clonidine has analgesic properties to which tolerance develops, is used to treat opiate withdrawal, has spinal and supraspinal actions and involves alpha 2 adrenoceptors.

The Pharmacokinetics of Clonidine

The pharmacokinetics of clonidine have proved difficult to investigate because its potency results in low tissue levels with consequent problems of detection. To overcome this problem early investigators used large doses of clonidine. This assumes that extrapolation to lower doses is valid.

In man clonidine is well absorbed orally. It is equipotent when given IV or orally although the rate of onset of action is much faster by the former route (Davies et al 1977). Plasma levels correlate well with sedation and reduced saliva production (Dollery et al 1976). But for hypotension the correlation only holds for plasma levels up to 3 ng/ml (Frisk-Holmberg & Paalzow 1979)(Reid et al 1980). However Frisk-Holmberg (1980), using continuous infusions to maintain a steady plasma concentration of clonidine, found that the decrease in blood pressure was progressive, showing that plasma concentration alone is not the only determinant of hypotensive activity. In man the reported plasma half lives are similar: 20 hrs (Rehbinder 1969, Rehbinder & Deckers 1969), 11 hrs (Davies et al 1977), 13 hrs (Dollery et al 1975), 11 hrs (Frisk-Holmberg & Paalzow 1979). Though a wide range is found between subjects studied between 6-24 hrs after administration of clonidine (Dollery et al 1975). The plasma T1/2s have led to twice or thrice daily dosing. As noted in the sections on sedation and salivation these unwanted effects appear most markedly shortly after oral administration, corresponding to the peak in plasma concentration. To reduce these

fluctuations a slow release preparation is available, Catapres PL Perlongets (Boehringer Ingelheim Ltd.), intended for administration once a day. Tolerance develops to the sedative action of clonidine and to reduce its occurence the dosage is built up slowly (Velasco et al 1976). Similarly to avoid withdrawal symptoms dosing is curtailed slowly. Excretion occurs in both urine and the faeces with 40-60% appearing in the urine unchanged and 15-30% in the faeces (Houston 1931) (Rehbinder & Deckers 1969) (Davies et al 1977).

Rehbinder (1969) and Rehbinder & Deckers (1969) using ^{14}C labelled clonidine found clonidine to be readily absorbed after oral administration in the dog, rat, monkey and man. In the rat peak plasma levels of radioactivity were found after one hour and declined with a half life of ten hours. In the other animals absorbtion was slower but the half lives were of a similar order of magnitude. Excretion was investigated by recovering C-14 from urine and faeces. Over 72 hrs urine accounted for between 65 (rat) and 83% (monkey) of the given dose with the remainder appearing in the faeces. Metabolites in the urine were separated chromatographically and detected autoradiographically. This exposed differences between the species studied in respect of metabolites and the perecentage of the original dose excreted unchanged. In the rat 58% was present unchanged in the urine with 22% as para hydroxy clonidine whilst in the monkey only 14% appeared as clonidine with the balance as one of six metabolites that did not include 6 hydroxy clonidine.

In a similar study (Cho & Curry 1969) using 500 ug/Kg IP in rats 40% of the C-14 was recovered from the urine over 72 hrs of which 35% was clonidine. The plasma T1/2 was 3.25 hrs whilst it was 2 hrs for the stomach and heart. This study also reported binding to plasma proteins of between 40-62% and to tissue homogenates 15-30%.

Jarrott & Spector (1978) 100 ug/Kg IV in the rat found T1/2 of 90 mins for plasma and 25 mins for the brain, with peak levels in the latter appearing after 2 mins. Four hydroxy clonidine (also known as St 666) was detected in the plasma after 60 mins and after 90 mins was present in a higher concentration than clondine. This metabolite was noted by (Rehbinder 1969) in urine. It is a potent alpha adrenoceptor stimulant.

Conway & Jarrott (1980) using a sensitive radioimmunassay for clonidine were able to use doses in the hypotensive range, 20 ug/Kg

IV in the rat. They found T 1/2 s to be similar for all tissues, around 1 hr although that of the corpus striatum in the brain was longer. As clonidine is lipophylic, movement between tissues should be easy and therefore T1/2s similar.

There are major differences between the T1/2s reported by authors for the rat which may be related to the period over which tissue levels were monitored and/or the different dose used. Clonidine is a lipophylic drug whose plasma levels drops very rapidly after bolus dosage. Therefore the initial distribution is likely to reflect the blood flow to the tissue or organ. Areas with a high blood flow might be expected to exhibit an initially high drug level which would decline as the pattern of distribution comes to reflect lipid content and specific binding. It follows that the T1/2s for different areas will vary in the first few minutes after administration but become more uniform later when changing drug levels reflect excretion and metabolism rather than redistribution. Nerve discharge in the locus coeruleus was briefly inhibited by clonidine IV, the inhibition lasted for only a few minutes (Svensson et al 1975). This probably follows the concentration of clonidine in the CNS, the brief inhibition occurring at a time when concentration reflects tissue blood flow and the rapid offset following redistribution to tissues with lower blood flows. If the rate of metabolism follows first order kinetics or is altered by the anaesthetics used the different T1/2s become explicable. Larger values would reflect larger doses if metabolism was rate limiting. Metabolic studies do not appear to have been undertaken with this in mind though Frisk-Holmberg & Paalzow (1979) refer to dose related alterations in the pharmacokinetics.

Clonidine is a basic drug with a pKa of 8.05 (Struyker Boudier et al 1974). It follows that at pH 7.4 18% is present in an unionized form. At this pH clonidine has an apparent partition coefficient of 3.0 (Hoefke et al 1975) or 9.3 (Jarrott et al 1979) in octanol/water or 4.95 between chloroform/water (Struyker Boudier 1974) and a true partition coefficient of 27. The true partition coefficient is independent of pH and only concerns the differential distribution of the unionized form of the drug. Therefore at the pH found in plasma clonidine is a lipophylic drug and as such would be expected to rapidly equilibrate across lipid barriers. It follows

that equilibration will occur across the kidney tubules and that the rate of excretion will be exponential, which is found. Further it would be expected that the rate of excretion will reflect urinary flow and pH. These do not appear to have been investigated but might explain the wide range of plasma T 1/2s found in man.

Lipophilicity is a requirement for movement from plasma into the CNS. In the absence of active secretion the blood brain barrier reduces penetration by ionized molecules and poorly lipophilic substances. It is not an absolute barrier but combined with removal of substances from the cerebral extracellular fluid by bulk CSF flow it leads to much lower CNS than plasma concentrations.

Timmermans et al (1977a,b) using clonidine and clonidine analogs found that the brain concentrations, measured at the time of maximum blood pressure fall, were proportional to the doses given IV. The slope of the brain/plasma concentration graph was linear and reflected the lipophilicity of each substance. The study concluded that "the brain concentration is a measure of the hypotensive effect". It may be developed as an argument for a central site of action, that the brain concentration determines the hypotensive effect. This is specious as the concentration at any time is likely to be dose dependent and as the hypotensive effect is similarly dose dependent the argument may be made for any tissue. Measuring the concentration at the time of maximal blood pressure fall does not enhance the case. Struyker-Boudier et al (1975) compared clonidine with St 666, finding that the latter is a potent alpha adrenceptor agonist but ineffective in lowering blood pressure when given IV. Applied by injection into the hypothalamus it has a similar potency to clonidine in reducing heart rate. The apparent partition coefficients are 4.95 for clonidine and 0.003 for St 666, suggesting that St 666 is unlikely to penetrate the blood brain barrier and explaining the discrepancy between IV and intrahypothalamic dosing. This is an argument for a central site of action for clonidine, assuming that alpha adrenoceptor stimulation is the mechanism. St 666 is an important metabolite of clonidine in the rat but is unlikely to have central actions. As an alpha adrenceptor agonist it may exert peripheral actions on pre and post synaptic receptors and contribute to the progressive recovery in arterial pressure seen with clonidine after a single IV dose. Jarrott and Spector (1978)

measured the plasma levels of St 666 and found appreciable levels 30 mins after clonidine. Conway and Jarrott (1980) found that the T1/2 for clonidine levels and the hypotensive effect were similar in the rat, however no account was taken of possible pressor actions of St 666.

Hoefke et al (1975) compared the partition coefficients of St 91, a clonidine analog, and clonidine with the hypotensive potency when given IV or ICV. St 91 has an apparent partition coefficient of 0.06 and given IV fails to reduce blood pressure, given ICV it is active. Comparison with clonidine, lipophilic and potent IV and ICV establishes the importance of lipophilicity for IV antihypertensive potency and points to a central site of action.

Overall lipophilicity is a requirement for IV potency. It is important to note that though lipophylic only about 2% of the clonidine given IV is present in the brain (Timmermans et al 1977, Conway & Jarrott 1980).

The Pharmacology Of Clonidine

This section examines pharmacological effects of clonidine. It is generally regarded as an alpha adrenoceptor agonist with a preferential alpha 2 action but a variety of other actions have been suggested: purinergic antagonist, uptake 2 blocker, histamine H2 agonist, alpha adrenergic antagonist and membrane stabiliser.

Alpha Adrenoceptor Interaction

1. Adrenoceptors

Adrenoceptors were divided into two groups, alpha and beta by Alhquist (1948) and by Lands et al (1967) into beta 1 and beta 2. More recently alpha receptors have been separated into alpha 1 and alpha 2 on the basis of agonist and antagonist potency. Starke et al (1975) using rabbit pulmomary artery strips found differences in pre and post synaptic adrenoceptor actions in a range of alpha adrenoceptor agonists: methoxamine and phenylephrine were preferential post synaptic agonists, noradrenaline, adrenaline and naphazoline were of similar potency and oxymetazoline, alpha methylnoradrenaline and tramazoline more potent on pre synaptic

receptors. Using antagonists Doxey et al (1976) found yohimbine and phentolamine more potent antagonists at presynaptic receptors and phenoxybenzamine and prazosin more active on postsynaptic receptors. The antagonist with the largest pre/post ratio is rauwolscine (alpha yohimbine) (Tanaka et al 1978).

Alpha adrenoceptors occur pre and postsynaptically. Presynaptic receptors serve to modulate transmitter release. The initial subdivision into two classes, alpha 1 and 2, coincided with the two locations but the discovery of postsynaptic alpha 2 receptors in the vasculature has invalidated this arrangement (Drew et al 1979), (Kobinger & Pichler 1981), (Timmermanns & Van Zwieten 1980a,b). This may be related to the two populations of excitatory receptors for noradrenaline found in arteriolar smooth muscle (Hirst & Neild 1980). Kobinger & Pichler (1981) found each type of alpha 2 adrenoceptor pre and postsynaptically.

Within the CNS two types of alpha adrenoceptors have been identified. Miach et al (1978), using ^{3}H-dihydroergotamine displacement from rat brain homogenates, demonstrated two binding sites with differential preferencies for prazosin and yohimbine. U'Prichard et al found two binding sites for prazosin and two separate binding sites were noted by Greenberg & Snyder (1977) and Peroutka et al (1977). Hammer et al (1980) identified two clonidine binding sites with Kds of 0.4 and 6.1 nM as did Vetulani et (1979), but Jarrott et al (1978) found only one with a Kd of 1.7 nM and U'Prichard et al (1979) located a high affinity site with a Kd of 0.4 nM. Hill plots indicate no cooperative binding. The binding at the two sites generally conforms with pharmacologically identified alpha adrenceptors found in the periphery. Binding studies alone are insufficient to establish the presence of pharmacologically active receptors, which further requires a physiological response. Anden et al (1976) provide functional evidence for two classes of central adrenoceptors and Aghajanian & Van der Maelen (1982), using ionophoresis of drugs close to neurones, clearly established the existence of central alpha 2 adrenoceptors. Anden et al (1976) found clonidine less potent in stimulating the hindlimb flexor reflex and potentiating the apomorphine induced locomotor activity than in reducing the alpha-methyl-tyrosine mediated disapperance of noradrenaline from the brain and spinal cord. The former occurred

with 0.4 mg/Kg IP and the latter at only 0.1 mg/Kg IP. Phenoxybenzamine and haloperidol were more effective than yohimbine and piperoxane at abolishing the functional effects whilst the potencies were reversed for the biochemical changes consequent on clonidine.

Central alpha adrenoceptors appear both presynaptically and postsynaptically, Helder et al (1981) found an alpha 2 adrenceptor mediated reduction in electrically evoked catecholamine release from cortical slices, a presynaptic effect. U'Prichard et al (1979) destroyed noradrenergic nerve terminals in the rat corpus striatum without reducing either alpha 1 or alpha 2 binding, indicative of a postsynaptic location for each in this area of the brain. Dausse et al (1982) in cerebral cortex found a 20% reduction in alpha 2 adrenceptors after lesioning with 6 hydroxydopamine whilst alpha 1 adrenoceptors increased slightly, locating alpha 2 pre and postsynaptically and limiting alpha 1 to postsynaptic sites. This pattern of distribution is the same as that commonly reported in the periphery, with alpha 1 appearing only post and alpha 2 at both locations. Presynaptic alpha adrenoceptors within the CNS are not confined to catecholamine-containing neurons and are seen on serotonergic nerve terminals (Maura et al 1982).

In the periphery alpha adrenoceptors of cardiovascular importance are found in the vasculature mediating vasoconstriction (alpha 1 & 2), in presynaptic nerve terminals in the autonomic nervous system reducing transmitter release (alpha 2) and in the myocardium causing a positive inotropic effect (receptor uncharacterized) (Williams et al 1978). The latter appears of little physiological importance, is antagonised by phentolamine and is distinct from B 1 adrenoceptor mediated inotropism.

Within the CNS alpha adrenoceptors are widely distributed and are discussed further in the section on the site of hypotensive action of clonidine, later in this chapter.

To establish that central and peripheral alpha adrenoceptors are the same would be of great use. A binding study using a homogenate of central and peripheral tissue and ^{3}H clonidine could be used. If only the two binding sites found with brain tissue were found in the mixed homogenate then it might be reasonably be concluded that the receptors are the same. This study does not

appear to have been undertaken.

2. Clonidine as an alpha adrenoceptor agonist.

In the isolated aorta, whole animal and cat nictitating membrane clonidine shows alpha adrenoceptor activity.

The action on the aorta is constrictor, similar to adrenaline and noradrenaline. It is sensitive to phenoxbenzamine (Constantine & McShane 1968) and phentolamine (Boissier et al 1968). However it is not a full agonist having a lower maximal effect than adrenaline and noradrenaline (Medgett et al 1978), a less steep drug response curve, shows greater receptor protection against phenoxybenzamine than adrenaline and reduced the constrictor response to a dose of adrenaline othrerwise able to evoke the maximal response. Phenoxybenzamine binds covalently to alpha adrenoceptors but binding is prevented if an agonist is present. The implication behind clonidine's greater protective role than adrenaline, despite a smaller constrictor action, is that it occupies the receptor but only stimulates it weakly, i.e. it acts as a partial agonist.

A bolus injection IV produces a brief pressor response in perfused limbs, intact and spinal animals, which IV phentolamine abolishes (Constantine and McShane 1969) showing it to involve alpha adrenoceptors. Immunosympathectomy (Zaimis 1968) and reserpinization (Autret et al 1971) do not diminish the pressor action which therefore does not involve catecholamine release. Low doses of clonidine, 20 ug/Kg, potentiate the pressor effect of IV adrenaline and noradrenaline, whilst doses greater than 100 ug/kg, reduce the pressor effect, revealing an intrinsic activity of less than one. The pressor effect in pithed rats is of short duration, following the rapid decline in plasma level seen with bolus administration (Docherty & McGrath 1980) and the peak effect depends upon the rapidity of the injection. It is assumed that the pressor effect involves alpha 1 adrenoceptors but the establishment of postsynaptic alpha 2 adrenoceptors (Drew et al 1979) in the vasculature necessitate a reappraisal.

Bentley et al (1977) noted the ability of prazosin (competitive alpha 1 adrenoceptor antagonist) to reduce noradrenaline induced contractions in human visceral arteries but its failure in peripheral arteries where phentolamine was efficacous (preferential

alpha 2 adrenoceptor antagonist, with some alpha 1 action). Using cats and pithed rats marked differences were seen in the effectiveness of each antagonist in reducing the vasoconstricting actions of phenylephrine, noradrenaline and nerve stimulation. Prazosin was ineffective with nerve stimulation, moderately effective with noradrenaline and potent with phenylaphrine, while phentolamine antagonized all three well. Clonidine in the pithed rat (Autret et al 1971) is less potent as a vasoconstrictor than noradrenaline but has a similar maximal response, phentolamine causes a parallel shift in the log dose response relationship, suggesting competitive antagonism. Clonidine is more potent at alpha 2 adrenoceptors, which are clearly established in the vasculature, but in the pithed rat very different time courses are found for actions on the vasculature and presynaptic inhibition (Docherty & McGrath 1980). Both should involve alpha 2 adrenoceptors and therefore have a similar time course. The central hypotensive action of clonidine is also attributed to alpha 2 adrenoceptor stimulation yet the hypotensive dose does not display a concomitant pressor action. In doses above therapeutic levels a pressor effect is seen Wing et al (1977) and at plasma levels above 3 ng/ml the correlation with hypotensive activity falls. But if both actions stem from a similar receptor a pressor action should be discernible. It could be that a peripheral action is masked by pressor action of circulating catecholamines, or that clonidine may act as an antagonist. Boissier et al (1968) in cats, dogs and rats found potentiation by low doses of clonidne of the pressor actions of adrenaline and noradrenaline but antagonism by high, showing a partial agonist action. Noradrenaline is slightly more potent, ratio 1.3, at alpha 1 than 2 adrenoceptors (Starke et al 1975) and active on alpha 2 at similar concentrations to clonidine. In the pithed rat clonidine (Autret et al 1971) appears as a full agonist, although competition with noradrenaline was not researched. In the pithed rat levels of catecholamines will be low and a maintained pressor effect of clonidine expected. It's absence needs to be explained. Fla-136, a clonidine analog, lowers blood prressure when applied ICV and like clonidine has its cardiovascular effects reduced by yohimbine ICV (Hamilton & Longman 1979). Given IV Fla-136 has no pressor actions which may be indicative of differences between central and

peripheral alpha adrenoceptors.

In a close arterial infusion tachyphylaxis to clonidine but not noradrenaline appeared (Zaimis 1968) a confusing finding not supported by later workers on the cardiovasclar system (Ruffolo et al 1977). Tachyphylaxis is seen with imidazole alpha agonists (clonidine, oxmetazoline etc) on the rat vas deferens and not with phenylethylamine alpha agonists (adrenaline, noradrenaline etc). Further, imidazole-induced tachyphylaxis does not extend to the phenylethylamines, suggesting that different sites of action are involved. Binding studies with dihydroazetepine reveal antagonism by imidazoles but potentiation with phenylethylamines. The pA_2 with phentolamine is similar for each group of agonists. The authors propose that each class of agonist acts at a separate location on the receptor and explain the similarity between pA_2s as occurring at a part of the receptor shared by both families of alpha agonists. Kobinger et al (1981) found tachyphylaxis to the vasoconstricting effect of clonidine in the perfused rat hindquarters preparation, cross tolerance was seen with noradrenaline. Although the repeated application of noradrenaline did not not lead to a diminution in response. Tachyphylaxis does not appear with the pressor response in the pithed rat and this discrepancy needs to be explained. Kobinger et al (1981) refer to loss of intrinsic tone in the hindlimb vasculature but do not explain why this is not apparent in the pithed rat. Zaimis (1968) found tachyphylaxis with clonidine after close arterial infusion and loss of intrinsic tone is unlikely in this preparation.

Presynaptic alpha 2 adrenoceptors reduce transmitter release from autonomic nerve terminals (Langer 1977, 1977), conversely antagonists increase release (Docherty & McGrath 1979). Clonidine is active at this site. Starke et al (1975) compared the reduction in ^{D3}H noradrenaline efflux after electrical stimulation of an isolated pulmonary artery with the postsynaptic constrictor action. Clonidine has a pre/postsynaptic agonist ratio of 6 and phenylephrine 0.03 a major discrepancy in the prevailing assumption of alpha adrenoceptor homogeneity. The PA_2 of phentolamine with a range of agonists also differed markedly pre and postsynaptically but not between agonists at each location, two receptor types. The net effect of an agonist on the constrictor response to nerve stimulation depends on the

pre/post ratio. If it has preferential presynaptic actions transmitter release will be reduced and so therefore will the postsynaptic response whilst the opposite holds if the ratio is reversed.

The action of clonidine on presynaptic receptors is concentration-dependent between 10pM and 10uM. The effect on transmitter release also varies with stimulation frequency. At low frequencies of nerve stimulation (less than 5 Hz) transmitter release is attenuated but above 5hz it is enhanced (Medgett et al 1978). This is used to establish clonidine as a partial agonist at this site. At low stimulation frequencies the concentration of noradrenaline would be low and clonidine stimulates presynaptic recptors thereby reducing transmitter release but at higher concentrations noradrenaline and clonidine compete for the receptor. If both had an intrinsic activity of one, greater stimulation would result with a progressive reduction in transmitter release per impulse, there finally being no difference between the control and clonidine treated preparation. The reported clonidine/noradrenaline ratios at the presynaptic alpha adrenoceptor are similar 1.2 (Berthelsen & Pettinger 1977) and 0.8 (Starke et al 1975).

Clonidine also reduces actylcholine release at parasympathetic nerve terminals (Werner et al 1972).

3. Clonidine as an alpha adrenoceptor antagonist.

Noradrenaline acting on alpha and beta receptors, increases cyclic AMP levels in rat cerebral slices. Clondine has no agonist action, 1-100uM, but reduces the evoked response to that resulting from beta stimulation at only 0.1uM. Phenoxybenzamine is also an antagonist but does not further enhance clonidine blockade, suggesting a common site of action (Skolnick & Daly 1975). In addition clonidine potentiates the effect of beta agonists.

In dispersed rat parotid cells adrenaline evokes potassium release. Clonidine does not and it reduces the level of response to adrenaline. In addition clonidine reduces dihydroergotamine binding to receptors in parotid cells, an action seen with other imidazoles (Davis & Maury 1978).

An excitatory effect of iontophoreticly applied noradrenaline on a bulbar cardiovascular neurones was blocked by iontophoreticly

applied clonidine which alone was inactive (Sharma et al 1978). The authors conclude "it is apparent that clonidine and noradrenaline act upon the same adrenergic receptors". No use of selective alpha or beta antagonists was made making the conclusion less authoratative, but it is nonetheless quoted as a case of alpha antagonism in the literature.

Human blood platelets aggregate when exposed to adrenaline or noradrenaline, while clonidine and yohimbine antagonise the response suggesting an action at a similar receptor. Vasopressin, 5HT and ADP also cause aggregation but for these agents clonidine is a potentiator, an action opposed by yohimbine (Grant & Scrutton 1979).

Clonidine can act as an antagonist to noradrenaline evoked vasoconstriction in the perfused rat hindquarters (Kobinger et al 1981). The concentration in the perfusate used to demonstrate this effect was 10 ug/ml, far higher than that encountered during antihypertensive therapy.

In each case clonidine appears to exhibit an alpha adrenoceptor antagonist action, but they could be interpreted as partial agonist actions. Clonidine potentiated the isoprenaline induced rise in cyclic AMP in the rat cerebral slices and platelet aggregation caused by non adrenergic agents. It is well established that clonidine has an intrinsic activity of less than unity producing a less than maximal response in some preparations and acting as an antagonist in the presence of more potent agonists. It is assumed that agonists only activate a small fraction of the available receptors when producing a maximal response, the idea of spare receptors. A partial agonist may produce a full response but needs to stimulate more receptors. With a smaller receptor pool the partial agonist will produce a submaximal response and a further reduction in receptor numbers may lead to no end organ response. In the presence of a agonist this appears as antagonism. Where there are agonists with similar actions but mediated through a different receptor the subthreshold stimulation is revealed by potentiation of the second agonist. This is seen with clonidine.

Uptake 2 Antagonism

Clonidine reduces noradrenaline uptake (Starke et al 1972, Katsuragi 1978). In a more detailed paper (Salt 1972) this was shown

to involve extra neuronal tissue (uptake 2) and no action on uptake 1 was reported. In view of the high concentration required, 50uM, a role in the hypotensive response seems unlikely.

Action As A Purinergic Antagonist

Stone & Taylor (1978a and 1978b), in two remarkably similar papers, found that the iontophoretic application of adenosine or adenosine-5-monophosphate reduced the frequency of discharge of neurons in the rat motorsensory cortex. Clonidine in doses having no overt action, antagonised this effect whilst failing to reduce the responses to iontophoretic adrenaline, 5 hydroxytryptamine or GABA which were depressant. The ability to antagonise the response to one group of agonists but not others having similar actions suggests that in this preparation at least clonidine functions as a purinergic antagonist. Purines have a role in reducing catecholamine release from nerve terminals acting through presynaptic receptors (Su 1975, Su & Tsuru 1978) but there appears to be no work on possible interactions with clonidine. It is hard to assess the importance of these results as the concentration of clonidine achieved during iontophoretic application is unknown, a problem with all iontophoretic experiments.

Local Anaesthetic Action

Clonidine is as potent as procaine in the guinea-pig wheal test (Hoefke & Kobinger 1966), slightly more potent in reducing conduction in the isolated frog sciatic nerve (Starke et al 1972) and more potent in the rabbit corneal reflex test. A concentration of approximately $1x10^{-4}M$ is required for these actions. At high concentrations and in the presence of burimamide, a histamine H2 antagonist, a negative chronotropic effect is seen on the isolated rabbit heart (Csongrady & Kobinger 1972) which is probably due to a local anaesthetic action.

Action As A Histamine H2 Agonist

In many tissues clonidine appears to act as an H2 agonist:

1. Clonidine 50 ug/Kg IP induced hypothermia in the conscious rat is reduced by ICV phentolamine 5-10ug and cimetidine 25-50ug but not by propranolol 50ug. Administered IV clonidine is ineffective

(Bugajski et al 1980). Noradrenaline ICV also causes hypothermia which is antagonized by phentolamine but not by cimetidine or propranolol. This suggests that hypothermia involves both central histamine H2 and alpha adrenergic receptors and that clonidine but not noradrenaline acts on both.

2. A positive inotropic action of both histamine and clonidine is seen on the isolated perfused rabbit heart (Csongrady and Kobinger 1974) that is antagonized by burimamide, a histamine H2 antagonist, the PA2 is similar with each agonist implicating a common receptor. Parsons (1978) shows similar actions on the guinea-pig atrium with histamine and clonidine, the latter is however much less potent and only able to exert 18% of the maximal response possible with histamine, making clonidine a partial agonist. To show an inotropic action Csongrady & Kobinger added 10ug of clonidine to an isolated perfused heart probably achieving a concentration greatly in excess of that required to lower blood pressure where 10 ug/Kg IV is effective.

3. On the isolated rat uterus cimetidine shows a similar PA2 against clonidine and histamine but clonidine has a low relative potency and functions as a partial agonist (Parsons 1978).

4. Clonidine $5x10^{-6}$M and histamine have a lipolytic action in isolated dog adipocytes and stimulate cyclic AMP production, cimetidine but not mepyramine, a histamine H1 blocker, shows antagonism (Berlan et al 1981). These actions are at variance with those of an alpha adrenoceptor agonist which exhibit anti-lipolytic properties on adipocytes.

5. Sastry & Philips (1977) showed that iontophoretic application of clonidine, noradrenaline and histamine depressed rat cerebral cortical neurons and that metiamide blocked the actions of clonidine and histamine but not noradrenaline. However in a further population of neurons clonidine was excitatory, histamine inactive and noradrenaline depressant, metiamide had no action. Sastry & Philips (1976) show histamine acting via H1 and H2 receptors and a divergence in the actions of histamine and clonidine may be

explicable in these terms, the ability of metiamide to antagonise clonidine but not noradrenaline implies an action at histamine H2 receptors.

6. Receptor binding studies (Timmermans et al 1980) show that clonidine reduces 3H prazosin, an alpha 2 adrenoceptor antagonist, binding in brain tissue but that cimetidine only interacts at high concentrations. This study only shows that cimetidine does not have alpha 2 adrenoceptor actions and says nothing about clonidine having a histamine H2 agonist action. Pilc et al (1979) found that cimetidine, metiamide and 4 methylhistamine, an H2 agonist, failed to reduce 3H clonidine binding to rat cerebral cortical receptors in concentrations less than $1x10^{-4}$M and that alpha 2 adrenoceptors accounted for the clonidine binding. As clonidine shows many examples of actions compatible with a histamine H2 agonist role the results of these binding studies are surprising and may reflect the artificial conditions under which these studies occur.

7. According to Audiger et al (1976) clonidine (100uM) increases cyclic AMP levels in guinea-pig brain via histamine H2 receptors but at lower concentrations has no effect (Skolnick & Daly 1975). Cyclic AMP levels are also increased in gastric tissue (Karppanen & Westermann 1973) using $1x10^{-3}$M clonidine which is antagonized by burimamide and in adipocytes (Berlan et al 1981) which is antagonized by cimetidine.

8. Histamine H2 receptor antagonists are reported to antagonise the antihypertensive actions of clonidine: a) (Karppanen et al 1976) metiamide (300 ug) ICV given before or after IV clonidine reduces the fall in blood pressure but alone has no action on the cardiovascular system, b) (Paakkari et al 1976) metiamide ICV competitively reduces the effect of clonidine ICV but given alone increases BP, c)Finch et al 1978 showed that ICV cimetidine and metiamide reduce the antihypertensive action of IV clonidine but had a hypertensive action in high doses and a hypotensive action in low doses, d) (Karppanen et al 1977) metiamide alone ICV raised BP and reduced the action of clonidine, e) (Borkowski & Finch 1978) metiamide and cimetidine ICV reduced the hypotensive action of

clonidine, f) (Frisk-Holmberg 1980) working on conscious rats in contrast to the workers mentioned above who used urethane anaesthetized animals found that IV cimetidine (2.5-5 mg/Kg) reduced the effect of low doses of clonidine. This paper is novel in that cimetidine is given IV when it is generally reported not to cross (Cross 1973) the blood brain barrier, that clonidine with cimetidine increased BP whilst cimetidine alone was inactive, a point not dwelt upon in the text, and that cimetidine given after the establishment of clondine-induced hypotension was inactive. Watkins et al (1980) using six normotensive human subjects showed no interaction between clonidine (0.2 mg) IV and cimetidine (300 mg) IV, doses compatible with those used by Frisk-Holmberg. The action of H2 antagonists does not alter the hypotensive response to ICV alpha methyldopamine (Finch et al 1978) and has not been tried against alpha methyldopa. As a further complication Finch & Hicks (1976) and Tadepelli & Mills (1978) found no evidence for a clonidine histamine H2 receptor interaction in the cat.

9. Gastric acid secretion is increased in anaesthetized animals by clonidine (Karppanen & Westermann 1973) (Walz & van Zwieten 1970) an action held in common with histamine. Walz & van Zwieten using rats and guinea pigs found that clonidine (10-1000ug/Kg) IV caused a short lasting increase in acid secretion. This is blocked by cimetidine (Parsons 1978) (Jennewein 1977) but not by phentolamine (Cheng et al 1981). In all these experiments clonidine was used in high doses, up to 5mg/Kg, IP whilst cimetidine was used in doses compatible with histamine H2 antagonism.

The proposition that clonidine has histamine H2 agonist properties is supported by clonidine's actions in a variety of preparations and the ability of histamine H2 antagonists to reduce the effectiveness of clonidine.

Cardiovascular changes attendant upon the ICV administration of histamine and histamine H2 agonists do not mimic those of clonidine (Finch & Hicks 1977, 1978) (Hicks 1978), being in contrast to clonidine pressor. The pressor action of dimaprit, a histamine H2 agonist, is antagonized by metiamide 400-880ug ICV (Ericksson 1980),

a dose sufficient to reduce the hypotensive action of clonidine. The premise that clonidine and dimaprit act as agonists through the same receptor is hard to reconcile with these results unless the capriciousness of the ICV administration is invoked, mentioned in more detail in the section on clonidine's site of action.

The cardiovascular effects of ICV H2 antagonists are variable being reported as pressor, depressor and inactive, (Finch et al 1978) the action is dose dependent being pressor at high doses and depressor at low doses.

Histamine H2 antagonists have actions not compatible with histamine blockade. Catecholamine release by burimamide and metiamide 2 mg/Kg IV was demonstrated (Brimblecombe et al 1976) in the chronically denervated nictitating membrane and a pressor response in the pithed rat (Ganellin & Owen 1977) involving the adrenals shown with metiamide, burimamide and two other histamine H2 antagonists after IV dosing in the range 2-16 mg/kg. The pressor effect was of short duration and probably related to the initial high plasma levels encountered after bolus administration, Frisk-Holmberg did not report a pressor response in conscious rats with IV cimetidine. When used to reduce the hypotensive effect of clonidine up to 800 ug/rat ICV are employed, as the volume of distribution is small it is not impossible that catecholamine-releasing concentrations are achieved within the brain. The 800 ug contrasts with 25-50 ug used to reduce the hypothermic action of clonidine (Bugajski et al 1980) possibly reflecting a different mechanism. There is evidence that metiamide acts as a presynaptic alpha adrenoceptor antagonist (Griffin et al 1978)(Doxey & Everitt 1979) in doses above 3mg/Kg IV. Cimetidine is reported to act as a narcotic antagonist in the guinea-pig ileum at concentrations of $1x10^{-4}$M.

Pilc et al (1979) report that high levels of cimetidine and metiamide reduce clonidine binding to cerebral tissue and given the large doses administered the unknown volume of distribution another mechanism not involving histamine receptors is available to explain the reduction of clonidine induced hypotension by histamine H2 antagonists.

Clonidine reduces the rate of central noradrenaline depletion after Fla-63, a dopamine B hydroxylase inhibitor. Metiamide

pretreatment did not alter this action of clonidine (Pugsley & Lippman 1979) suggesting that H2 receptors are not involved. This study is marred by the failure to report the effect of clonidine and the interaction with metiamide on blood pressure.

However even if it is demonstrated that histamine H2 antagonists are acting in their named role this does not imply that antagonism towards clonidine occurs at the level of the receptor, in a complex system involving many neurons and receptors and containing a feedback loops drugs have a superfluity of possible sites of action and that two drugs exert opposing effects need not imply an interaction at the molecular level.

Clonidine appears to exhibit a histamine H2 agonist action but only when administered in high doses or concentrations, the action of histamine H2 antagonists in reversing clonidine's hypotensive effect is well established but reasonable doubt exists as to the mechanism necessitating further research.

Overall it appears that clonidine's pharmacological actions are concentration dependent. At low concentrations it acts as an alpha 2 partial agonist and as the concentration rises alpha 1 partial agonist actions appear. Further increases lead to histamine H2 agonist/partial agonist properties, purinergic antagonism, uptake 2 antagonism and finally local anaesthetic properties. Therefore when investigating the site and mechanism of the hypotensive effect care must be taken to limit the concentrations used, to avoid results and conclusions which though experimentally based are artifacts, real in themselves but unrelated to the problem under investigation. Lakdawala et al (1980) studied mast cell degranulation caused by clonidine, concentrations of 10-80 ug/ml caused histamine release yet 2 ng/ml is the concentration achieved in vivo, clearly this study is unrelated to any clinical problem.The dose dependance of responses is clearly seen in the work undertaken on acid secretion where the quantity of clonidine used varied from 0.005 to 5mg/Kg and both secretory and antisecretory actions may be seen. As the dose is increased superimposition of actions occur, the experiments on acid secretion all occurred with doses of clonidine that would alter blood pressure yet the effect of this was rarely mentioned and not

controlled for in the majority of studies.

The pharmacological data suggests that clonidine acts at alpha 2 adrenoceptors as a partial agonist. As a partial agonist the result of its application depends upon the concentration of the agonist already present. In the absence of an agonist clonidine produces dose dependent stimulation with a maximum lower than that of a full agonist. With high concentrations of agonist present the addition of clonidine has an antagonist effect, reducing the level of stimulation to that achievable with clonidine alone. It follows that clonidine can act differently on systems containing alpha 2 adrenoceptors. The use of analogs with differing partial agonist strength may be fruitful in the separation of hypotensive activity from sedative and analgesic effects. Further complications involve the intrinsic activity of the native agonist and spare receptors. To develop clonidine analogs with differing ratios of sedative/hypotensive/analgesic actions a detailed knowledge of the site of action and its pharmacology are necessary.

Clonidine And Plasma Renin Activity

Clonidine reduces plasma renin and the reduction correlates well with the fall in blood pressure (Niarchos et al 1978). Though in low renin hypertensive patients clonidine lowered arterial pressure without a reduction in PRA (plasma renin activity) and was effective in patients in whom saralasin was ineffective. Reports exist of increased PRA during clonidine hypotension (Thananopavarn et al 1976). Acutely PRA may increase but over a longer period a reduction or no change is seen (Salvetti et al 1973). PRA increases when arterial pressure is reduced therefore the maintenance of PRA in the face of a reduction in arterial pressure represents a change in this relationship.

In the conscious rat Pals (1975) could only obtain a reduction in arterial pressure after sodium depletion which raised plasma renin activity.

A central mechanism is involved. After spinal cord transection an increase in PRA appears rather than the reduction seen in an intact animal (Ganong et al 1978). A direct inhibitory action was reported by Pettinger et al (1976), after ganglion blockade. The failure to maintain renal perfusion pressure devalues this study.

When perfusion pressure is maintained an increase follows clonidine in ganglion blocked animals (Nolan & Reid 1977).

A reduction in plasma renin occurs within 15 minutes of IV injected clonidine in the dog and the reduction increases over the following thirty minutes (Ganong et al 1978). In many experiments arterial pressure is seen to fall dramatically after clonidine, often within seconds. It is unlikely that a a reduction in plasma renin occurs within this brief period although it may contribute over a longer period.

The Site Of Clonidine's Hypotensive Action

A definition of site of action of a drug is "the point where the local concentration determines the duration and intensity of the response".

A large number of sites have been proposed for clonidine's hypotensive and bradycardic action. These include the ventral surface of the brainstem, hypothalamus, spinal cord, presynaptic receptors on sympathetic efferent nerves, forebrain and reticular formation.

A Critique Of Methodology

Various methods have been used in the search for sites of action. Those most frequently employed are local administration, lesioning, biochemical and pharmacological.

Lesioning: Destroying part of the system under study and its effect on the drug induced response is useful when the operation of the lesioned area is well understood, including its inputs, outputs and function but is otherwise confusing. Removal of areas rostral to the pons alters the response to clonidine but this does not imply that the site of action is located in the lesioned area. A potential site of action is presynaptically on projections from the hypothalamus to the intermediolateral areas of the spinal cord. The lesion would abolish the hypotensive action of clonidine by removing descending neuronal activity. A site of action in the medulla that projected to the hypothalamus and hence to the spinal cord would also result in a loss of hypotensive activity after lesioning.

Therefore interpretation of lesioning experiments is difficult. A further problem involves ascertaining the size of a lesion, damage may extend further than the anatomical limits or if performed chronically (6-hydroxydopamine or immunosympathectomy) denervation supersensitivity will develope making interpretation difficult if not impossible.

When a well understood lesion is employed eg deafferentation of baroreceptors, useful results can be obtained.

Biochemical Methods: areas whose metabolism alters after clonidine administration are potential sites of action. But clonidine has multiple central actions, reduction in gastric secretion, reduction in saliva production, sedation, analgesia and hypotension. Therefore a change in metabolism is not necessarily indicative of the hypotensive site. Further an action at a point in a neuronal network will alter the metabolism at all sites peripheral to it which increases the areas where changes occur. In the cardiovascular system alterations in blood pressure lead to a change in afferent neuronal input which will have metabolic consequences. Taking a simplistic view of the cardiovascular system as a number of sequentially connected neuronal groups with afferent input and efferent output, clonidine will cause changes on the efferent side of its site of action consistent with reduced efferent activity whilst the consequent reduced blood pressure will alter afferent input in the direction that in the absence of clonidine would lead to an increase in sympathetic tone. Changes might be expected to occur in all areas associated with the cardiovascular system thus making interpretation of the results difficult.

The employment of adequate controls to compensate for alterations in afferent input becomes of great importance. They might include the use of peripheral vasoconstrictors and dilators. Vasodilators would alter afferent input in a similar manner to clonidine whilst efferent nerve activity would be expected to increase, the opposite of the change found with clonidine. Vasoconstrictors alter afferent input in a manner not found with clonidine and efferent activity would be expected to decrease. Thus the metabolic effects of clonidine on the cardiovascular system ought to resemble on the afferent side those found with a

vasodilator and on the efferent side those found with a vasoconstrictor. The area where the changes switch from one mode to the other is then likely to be the site of action.

Another approach would be to infuse a vasoconstrictor to reestablish the original blood pressure, leaving afferent input unchanged but output altered by clonidine, the site of action then comes from a comparison with an untreated control animal. Or afferent inputs could be removed: cut the vagi, destroy the baroreceptors and chemoreceptors. This would leave afferent input unchanged after clonidine except for afferents running in sympathetic nerves.

Clearly metabolic studies are difficult to conduct usefully.

Pharmacological: having tentatively identified clonidine as an alpha 2 adrenoceptor agonist it follows that the site of action will contain the appropriate receptor. Therefore the class of sites with the receptor will contain that for hypotensive action. Young & Kuhar (1979,1980,1981) used a combination of autoradiographic and receptor-binding techniques to locate central alpha adrenoceptors in the rat.

Location of Alpha 2 Adrenoceptors

Thalamus Hypothalamus

high

supraotic nucleus

arcuate nucleus

dorsal medial nucleus of hypothalamus

periventricular areas

Hindbrain

high

locus coeruleus

nucleus tractus solitarius

nucleus commissuralis

nucleus raphe pallidus

elevated

cochlar nucleus

parts of floor of fourth ventricle

substantia gelatinosa of spinal trigeminal nucleus

Spinal Cord

low even levels in the grey matter, moderate in lamina 2.
only cervical spinal cord studied.

However the number of activated receptors required to produce a response may be very small, and the site of action contain only a few neurons making the number of receptors involved too small for identification autoradiographically.

Local Administration: it follows from the definition used for site of action that local administration is a useful approach to locating the site of action. The main problems are establishing the spread and concentration after administration. With clonidine the diversity and concentration dependent pharmacological actions increase the importance of establishing both spread and concentration.

A limited body of work exists on the quantitative aspects of microinjection. Rech & Domino (1959) found that a volume of 10 ul causes disruption of the injection site and that spread of the injectate up the cannula track occurred while Myers (1966) found that the movement of injectate from the injection site was dependent upon the volume used, molecular wt, solubility. Myers et al (1971) noted variations in the distribution, metabolism and entry into the

vasodilator and on the efferent side those found with a vasoconstrictor. The area where the changes switch from one mode to the other is then likely to be the site of action.

Another approach would be to infuse a vasoconstrictor to reestablish the original blood pressure, leaving afferent input unchanged but output altered by clonidine, the site of action then comes from a comparison with an untreated control animal. Or afferent inputs could be removed: cut the vagi, destroy the baroreceptors and chemoreceptors. This would leave afferent input unchanged after clonidine except for afferents running in sympathetic nerves.

Clearly metabolic studies are difficult to conduct usefully.

Pharmacological: having tentatively identified clonidine as an alpha 2 adrenoceptor agonist it follows that the site of action will contain the appropriate receptor. Therefore the class of sites with the receptor will contain that for hypotensive action. Young & Kuhar (1979,1980,1981) used a combination of autoradiographic and receptor-binding techniques to locate central alpha adrenoceptors in the rat.

Location and Concentration of Alpha 2 Adrenoceptors

Thalamus hypothalamus:	high	supraotic nucleus
		arcuate nucleus
		dorsal medial nucleus of hypothalamus
		periventricular areas
Hindbrain:	high	locus coeruleus
		nucleus commissuralis
		nucleus raphe pallidus
	elevated	cochlar nucleus
		parts of floor of fourth ventricle
		substantia gelatinosa of spinal trigeminal nucleus
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or metabolism. It then follows that work involving microinjection is potentially flawed.

Microionophoresis applies drugs to discrete areas but the problem of estimating the local concentration remains, this is only partially offset by the ability to apply a range of doses. The response of only a single neuron is recorded and there are no attendant changes in blood pressure and heart rate. This makes characterization of the neuron critical, although it is possible to find changes in action potential frequency corresponding to alterations in afferent input it is not possible unequivocably to state that the neuron alters efferent nerve activity. The pH of the solution and effects of different salts of the drugs used pose further problems. Finding changes in discharge pattern after IV drug application encounters the problems outlined in the section on biochemical methods, altered afferent discharge. Neuronal recording and iontophoresis are useful in studying identified sites of action when the underlying pharmacology is of interest but are not useful techniques for identifying potential sites of action.

Peripheral Action

A reduction in transmitter release by sympathetic postganglionic neurons, diminished vascular reactivity and stimulation of baroreceptors have been proposed as mechanisms for the hypotensive and bradycardic affects of clonidine.

Zaimis (1968) and Zaimis & Hanington (1969) found reduced responsiveness to exogenous vasoactive agents after chronic clonidine administration. In three cats treated for a week the vasoconstrictor response to adrenaline and noradrenaline were attenuated and the dilator effect of isoprenaline similarly reduced. Differences between the three animals used appear to be large and no mention was made of the effect of clonidine on blood presssure and heart rate. Lombardi et al (1976), with a low IV dose of clonidine, noted a reduction in the local vasoconstrictor response to lumbar sympathetic stimulation and a reduction in the pressor response to femoral nerve stimulation. These occurred without alterations in the spontaneous and evoked sympathetic discharge, measured at T3. This is indicative of a peripheral mechanism operating after low doses of clonidine. Shaw et al (1971) found a residual fall in peripheral

resistance after autonomic blockade, with phenoxybenzamine, atropine and propranolol. This was interpreted as a direct vasodilator action of clonidine. In other studies clonidine was found to enhance the action of vasoconstrictors when applied in submaximal doses (Kozelka et al 1980). When given in large amounts clonidine antagonized the action of catecholamines (Kobinger et al 1981) (Boissier et al 1968). Commonly antihypertensive therapy increases the responsiveness of the vasculature making the work of Zaimis more interesting.

An increase in the discharge in baroreceptor afferents is reported [Aars (1972), Sleight et al (1975), Korner et al (1978), Laubie et al (1976)]. Aars (1972) associated increased baroreceptor discharge with an increase in the diameter of the aorta since the relationship between the two was unaltered after clonidine. Dilatation of the aorta appeared minutes after a reduction in renal sympathetic activity. If reduced renal nerve activity is taken as an indicator of sympathetic tone at the aorta the failure of the two events to coincide shows that they are not related causally. The reduction in efferent sympathetic nerve activity is therefore not caused by the dilatation of the aorta and altered baroreceptor discharge. However the reduction in sympathetic nerve activity is progressive and varies between nerves (Krier et al 1979) reducing the importance of a temporal separation between aortic dilatation and reduced renal nerve activity. In vitro clonidine contracts aortic strips, acting as a partial agonist in reducing the action of adrenaline and noradrenaline an explanation for the in vivo action. Antagonism to noradrenaline is also seen in the human saphenous vein with concentrations above those associated with hypotension, 20 ng/ml, lower levels were not employed (Coupar & Kirby 1972). Piperoxan, an alpha adrenoceptor antagonist, dilates the aorta preventing further dilation by clonidine, supporting the partial agonist idea. Noradrenaline given after clonidine is still able to constrict the aorta. Korner et al (1974) found baroreceptor discharge increased over a range of mean blood pressures with a high, 20 ug/Kg IV, but not lower doses of clonidine. However sectioning the baroreceptor nerves (Schmitt et al 1968) (Laubie et al 1976) and lesioning of the NTS does not abolish the hypotensive action of clonidine. Administration of clonidine into the carotid

artery does not increase sinus nerve discharge and is no more potent than IV administration in lowering blood pressure (Constantine & McShane 1968). Yet this method of application would be expected to reveal any direct effects of clonidine on carotid tone or directly on the baroreceptors. Administration of clonidine IVert increaesed baroreceptor discharge (Laubie et al 1976) again arguing against a direct action on the baroreceptors. It is possible that the enhancement of baroreceptor discharge involves efferent nerve activity though the appropriate experiments have not been undertaken. Increased baroreceptor discharge follows clonidine administration but this is only a contributory hypotensive mechanism, evidence exists for both a direct and indirect enhancement of acivity.

Presynaptic receptors stimulation by clonidine reduces transmitter release. The physiological role of presynaptic receptors has been questioned (Chen & Kalsner 1979) and a number of imidazoles that are effective presynaptically and do not cross the blood brain barrier fail to lower blood pressure when given IV, making the case for the unimportance of this mechanism, (Pichler & Kobinger 1978). Fla 136, a clonidine analog, with no presynaptic action lowers blood pressure after oral administration to rats (Hamilton & Longman 1980), again suggesting that peripheral actions are unimportant.

No action on sympathetic ganglia are reported (Schmitt et al 1968).

Indications For A Hypotensive Action Involving The CNS

Cross-circulation experiments and injection by intravertebral and ICV routes establish a strong case for a central site of action. The lack of hypotensive effect of agents that have similar pharmacological actions to clonidine but do not pass the blood brain barrier strengthens the case as does the reduction in sympathetic nerve activity that follows clonidine treatment. Activity rises when a peripherally acting agent is employed to reduce blood pressure, mediated through the baroreceptor arc.

Infusion of clonidine into the vertebral artey in both dogs (Constantine & McShane 1968) (Sattler & Van Zwieten 1967) and cats (Sattler & Van Zwieten 1967), with doses that IV are ineffective or

of greatly reduced efficacy, leads rapidly to a fall in blood pressure and heart rate. The immediate pressor response that normally accompanies IV dosing is not apparent with IVert though a pressor action is seen when 200 ug is administered by this route. It is preceeded by a brief hypotension. After the hypertensive episode a sustained hypotension is seen. Intravertebral clonidine reduces the resistance of a perfused brachial artery but with a denervated paw a delayed vasoconstrictor effect appears (Constantine & McShane 1968).

A cerebral cross-circulation preparation (Sherman et al 1968) indicated that hypotension and bradycardia are centrally mediated. Clonidine given to the donor dog led to a brief rise in the cerebral perfusion pressure and a fall in blood pressure and heart rate in the recipient. Removal of baroreceptor input in the recipient increased the hypotensive effect but deafferentation increased the pre clonidine blood pressure. Vagotomy did not prevent the bradycardia revealing a sympathetic component. In the recipient dog clonidine can only act within the brain and the concentration of clonidine achieved will be similar to that in the donor animal. Unfortunately the authors did not compare the cardiovascular responses of the two animals, this might have indicated hypotensive actions outside the brain.

Reduced sympathetic nerve activity is reported by many authors (Schmitt et al 1968, Kobinger & Pichler 1976, Klupp et al 1970). The reduction appears within seconds of an IV dose of clonidine and slightly precedes the attendant hypotension and bradycardia (Schmitt et al 1968). In a number of animals respiratory modulation of efferent sympathetic discharge was more resistant to clonidine than the continuous background activity (Schmitt et al 1968). The magnitude of the reduction is dose dependent (Klupp et al 1970). However not all sympathetic innervations are affected equally (Krier et al 1979). Postganglionic efferents in the hypogastric nerve running to the bladder and renal nerve were depressed by low IV doses of clonidine whereas the lumbar colonic nerves were resistant to high doses. Further renal efferents could be silenced with clonidine yet in the hypogastric nerve 20-50% of the pre-clonidine activity remained in the face of large doses, 100-200 ug/Kg. Viscero sympathetic reflexes in the colonic lumbar nerve were resistant to

clonidine yet those appearing in the renal and hypogastric were sensitive to low levels of clonidine. Intestinal sympathetic discharge appears independent of bulbospinal control possibly accounting for the minimal reaction to clonidine. In man muscle sympathetic activity responded unpredictably and an increase or decrease could follow a clonidine induced fall in blood pressure (Frisk-Holmberg & Wallin 1979). Failure to obtain a uniform response may have involved recording from an amalgam of muscle vasodilator and vasoconstrictor fibres. Alternatively the new level of discharge represents a shift in the relationship between blood pressure and nerve activity, discharge is lower after clonidine than it would have otherwise have been at the new reduced blood pressure. The action of clonidine in reducing sympathetic tone and catecholamine release is used as a clinical test to separate between essential hypertension and phaeochromocytoma when plasma catecholamine concentrations are high. Clonidine acts on sympathetic nerves but not on the catecholamine secreting tumour and does not reduce plasmsa levels in the latter case, the basis of the test (Bravo et al 1981).

ICV administration lowers blood pressure and heart rate (Schmitt & Schmitt 1969, Kobinger & Walland 1967, Borkowski & Finch 1979, Bolme et al 1975, Orma et al 1980). The active dose is generally lower than required IV but the speed of onset of the hypotension is much reduced, appearing progressively over tens of minutes. ICV administration is associated with a reduction in sympathetic nerve activity and bradycardia. Further it is sensitive to pretreatments with alpha antagonists. ICV administration limits the spread of drug and as it is active at levels devoid of IV activity a case is made for a central site of action. Clonidine acts at a central site and ICV administration provides easy access accounting for the potency of this route. However emesis occurs with ICV clonidine, an effect never seen with IV (Finch 1974,1975). Presumably a response to a concentration, in excess of that seen after IV administration, acting on a periventricular site. Different responses attend the administration of clondine by IV or IVert after catcholamine depletion (Dollery & Reid 1974). The cardiovascular effects of IV clonidine were reduced but those following ICV were abolished. The differing sensitivities indicate that clonidine does

not have the same actions when applied by the two routes, attributing the residual IV action to peripheral action probably overstates their importance. The emetic action of ICV clonidine indicates the problems with localized dosing. The progressive nature of the hyptotension is also interesting, does it represent accumulation of drug at a site on the edge of the ventricles or movement through the ventricular system to a site of action ?.

The case for a central action in the hypotensive response to clonidine is overwhelming and a much effort has been made at further localization.

Biochemical Consequences

Clonidine in antihypertensive doses has no effect on noradrenaline, dopamine or 5HT concentrations in the central nervous system (Anden et al 1970, Laverty & Taylor 1969, Anden et al 1976).

When noradrenaline synthesis is prevented, by giving alpha methyl tyrosine, depletion results. Clonidine reduces the rate of depletion and the rate of synthesis (Anden et al 1976). Similarly the T1/2 of tritium, after loading catecholamine nerves with ^{3}H noradrenaline, was increased by clonidine, 20 ug/Kg IV, in all regions except the hypothalamus (Reid 1975). Further evidence for reduced noradrenaline turnover is the reduction in noradrenaline metabolites in CSF and reduced tyrosine hydroxlase levels, the latter after one week of clonidine treatment (Reid 1975).

Clonidine reduces catecholamine and 5HT turnover in doses lowering blood pressure making a prima facie case for the involvement of these metabolic changes in the hypotensive response, so does their increased turnover during clonidine withdrawal (Svensson & Strombon 1977). The implications for the mechanism of action are unclear (see discussion in earlier section on methodology). These studies did not compensate for the effect of altered blood pressure on catecholamine turnover, sectioning the buffer nerves increases noradrenaline turnover in the spinal cord (Chalmers & Wurtman 1971). Spatial discrimination is poor since large sections of brain were used in the catecholamine assays, therefore differential actions on tracts or nuclei within the sections will be missed. Further clonidine has a number of actions at low doses not involving the cardiovascular system and biochemical

correlates of these are likely to appear.

In rats clonidine reduces adrenaline turnover in the medulla oblongata without afffecting noradrenaline, the reverse occurs in the hypothalamus (Bolme 1980). Central adrenergic mechanisms are being increasingly considered in cardiovascular control.

Mechanism Of Bradycardia

Bradycardia invovles both the vagal and sympathetic nervous systems. Pretreatment with propranolol or atropine reduces the magnitude of clonidine evoked bradycardia but does not abolish it, showing that it is mediated through both the parasympathetic and sympathetic nervous system. The location of vagal preganglionic cell bodies in the nucleus ambiguus, cuneate nucleus and dorsal motor nucleus of the vagus requires that clonidine acts at a supraspinal site when causing bradycardia through a vagal action.

Clonidine reduces the cardioinhibitory effects of vagal stimulation through an action on presynaptic receptors. However there is one report of enhanced responsiveness of vagal efferents (Lisander & Wennergren 1979). The mechanism was not pursued.

Action On Baroreflex Arc

clonidine reduces both mean arterial pressure and heart rate. The normal operation of the baroreflex arc leads to a compensatory increase in heart rate when blood pressure is reduced therefore the appearance of bradycardia accompanied by a reduction in arterial pressure indicates that clonidine has an action on heart rate in addition to its hypotensive action.

After clonidine the slope of the heart rate/arterial pressure relationship is altered, favouring a greater fall in heart rate for a set change in in arterial pressure (Sleight et al 1975, Sleight & West 1975, Korner1979).

In conscious rabbits Korner et al (1974) found a baroreceptor dependent bradycardia involving vagal excitation and sympathetic inhibition, a baroreflex independent bradycardia involving only sympathetic efferents was also noted.

The increased responsiveness of the baroreflex arc can occur before a reduction in arterial pressure or a change in sympathetic nerve activity appear (Haeusler 1974). Clonidine 1-3 ug/Kg increased

the response to sinus nerve stimulation.

A temporal separation between alterations in the response to bilateral carotid occlusion and the fall in blood pressure were reported by (Tadepelli & Mills 1978).

The enhancement of the baroreflex arc has peripheral components, described in the section on peripheral sites of action for clonidine, increasing baroreceptor discharge. An action involving atrial receptors leading to a fall in heart rate was reported (Lisander & Wennergren 1979). This appeared in the spinal dog and had more to do with the pressor action of clonidine increasing left atrial pressure than the pressure independent activation reported for carotid baroreceptors. In clinical use left atrial pressure falls and the involvement of atrial receptors in clonidine invoked bradycardia is an artifact of the spinal preparation.

Vagal bradycardia is widely reported to be enhanced by clonidine (Kobinger & Pichler 1972) and depends on intact baroreceptor afferents. Alternatively enhancement of the vagal response by clonidine can be seen with sinus nerve stimulation (Laubie et al 1976, Haeusler 1974). This occurs when clonidine is administered IVert and therefore probably involves a medullary structure. The response to electrical stimulation of the nucleus ambiguus is not altered by IVert clonidine suggesting that it is not the site of action (Laubie et al 1976).

The nucleus of the tractus solitarius, the first relay for baroreceptor afferents, has been considered as a site of action for clonidine. Its acute destruction abolishes the vagally mediated bradycardia seen with clonidine but in chronically lesioned dogs vagal bradycardia again follows clonidine injection (Schmitt & Laubie 1979). The reduction in heart rate seen with electrical stimulation of the NTS is not altered by clonidine administered IVert.

It appears that the vagal bradyacardia seen after clonidine requires the existence of the NTS and the baroreceptor inputs. In the NTS catecholamines reduce heart rate and arterial pressure (Reis et al 1979, de Jong et al 1979).

Clonidine reduces the pressor response to bilateral carotid occlusion but not in midcollicular decerebrate cats or when

clonidine is confined to supracollicular areas (Tadepelli & Mills). Further the sympathetic response to increased and decreased baroreceptor input was depressed. The authors reasonably suggest that this is not an enhancement of the baroreflex arc. Clonidine was administered by a variety of routes into the ventricular system and access to the NTS should have been good. When clonidine was confined to supracollicular structures a reduced but still marked fall in blood pressure followed but without alterations in the response to bilateral carotid occlusion. This was novel since it is widely reported that IC administration of clonidine does not reduce arterial pressure. It is possible that the response is due to high concentrations of clonidine resulting from local administration and does not represent the normal action of the drug.

The IVert administration alpha 2 adrenoceptor antagonists enhances the response to carotid occlusion (Constantine et al 1982) an action reversing that of clonidine.

Overall clonidine enhances vagal bradycardia probably through an action within the NTS. After placing a lesion at this site clonidine is still able to lower blood pressure and heart rate through the sympathetic nervous system suggesting that more than one site of central action may be involved.

Central Catecholamines And Clonidine's Cardiovascular Actions

Depletion of central and peripheral catecholamines with reserpine and alpha-methyltyrosine does not abolish the vagally mediated bradycardia and the reduction in splanchnic nerve activity seen after IV clonidine, though ICV clonidine was ineffective (Kobinger & Pichler 1974,1976). A fall in blood pressure is prevented by the lack of peripheral catecholamines. A difference in IV and ICV actions is also reported by (Dollery & Reid 1973). Catecholamine depletion was restricted to the brain by applying 6 OHDA ICV, resting heart rate and arterial blood pressure were similar to control animals. The fall in heart rate and blood pressure after IV clonidine were much reduced and the actions of ICV clonidine abolished in 6 OHDA pretreated animals.

These results are equivocal on the role of central catecholamines. Complete abolition of the actions of clonidine would appear if its effect was mediated through noradrenergic neurons,

either an action on presynaptic receptors reducing tranmitter release or postsynaptic effect leading to catecholamine release. A partial reduction short of abolition suggests that central catecholamines have a role in the cardiovascular response to clonidine. A complication is the denervation supersensitivity seen after 6-OHDA (Ungerstedt 1971) which makes interpreting the reduced effect of IV clonidine more difficult.

The difference in the susceptibility of IV and ICV clonidine implies that the two mechanisms of action are different, see section ICV dosing in "Indications for a hypotensive action involving the CNS".

Ionophoretic Studies

Cedarbaum & Aghajanian (1977) found that ionophoretic clonidine reduced the rate of discharge of locus coeruleus neurons and in this action was more potent than noradrenaline. A later study (Aghajanian & Van der Maelen 1982) related the depressant effect of clonidine to hyperpolarization of the cell membrane through the activation of alpha 2 adrenoceptors.A depressant action of clonidine on locus coeruleus neurons was also noted by (Svensson et al 1975).

Sharma et al (1978) looking at bulbar cardiovascular neurons in the decerebrate cat noted opposing actions of clonidine and noradrenaline, clonidine reduced the firing of 20 from a population of twenty seven neurons all located within 2-3mm of the dorsal surface of the medulla.

Champagnat et al (1979) saw a common action for noradrenaline, adrenaline, isoprenaline and clonidine on bulbar respiratory neurones.

Anderson & Stone (1977) ionophoretically applied clonidine and noradrenaline to neurons in the medullary reticular formation and found a common action in 79% of their population. Clonidine depressed the firing of 61% of the neurons tested and had a longer lasting action than noradrenaline, this was also noted by (Cedarbaum and Aghajanian 1977).

A number of iontophoretic studies have been undertaken but offer little insight into the hypotensive site of action of clonidine. The Aghajanian & Van der Maelen (1982) study illustrates the utility of iontophoretic experiments, showing the mechanism of

action of clonidine in a well defined system but the studies are hard to relate to the hypotensive site of action of clonidine. The relevance of the iontophoretic study is brought into doubt by Svensson et al (1975). A dose of clonidine was administered IV whilst recording from LC neurons, a brief depression in firing followed. However the dose was in the hypotensive range and a prolonged reduction in blood pressure would be anticipated, not the brief response noted in neuronal firing. The action on blood pressure was not mentioned and a further complication is the effect of altered arterial pressure on LC firing, also not mentioned in this study.

Further Localization Of Central Site Of Action

Forebrain

Tadepelli & Mills (1978) and Klevans et al (1973) suggest a role for the forebrain in the clonidine-induced reduction in heart rate and blood pressure. Klevans et al evoked tachycardia and an increase in blood pressure by stimulating the medullary reticular formation, clonidine reduced the magnitude of both. Decerebration, at the midcollicular level, increased the magnitude of the stimulation evoked response but when undertaken in the absence of clonidine was ineffective. This does not necessarily require that clonidine act in the forebrain, only that a pathway stimulated by clonidine involves structures rostral to the pons. Tadepelli & Mills compared the responses to clonidine in midcollicular decerebrate, perfused third ventricle and brain intact anaesthetized cats. The perfused third ventricle preparation involved the cannulation of the aqueduct between the third and fourth ventricle, so that when clonidine is administered into the third ventricle its spread is limited to supracollicular areas. In the decerebrate and brain intact cats clonidine was injected into the third ventricle, giving access to the ventricular system. The brain intact cat produced the greatest reduction in blood pressure and heart rate. Similarly the reduction in the response to bilateral carotid occlusion was also greatest in the intact animal. In the animals where the spread of clonidine was restricted blood pressure and heart rate were reduced substantially but the consequencies of bilateral carotid occlusion

changed, heart rate increased and blood pressure failed to respond. It then appears that an intact brain is required for the full actions of clonidine. However in each preparation the same dose of clondine, 25 ug, was given, despite a reduction in the volume of distribution. It follows that in the areas to which clonidine had access the concentration of clonidine will exceed that found in the brain intact animal, making comparisons questionable.

A pressor action of clonidine in the forebrain is widely supported. Bousquet & Guertzenstein (1973) obtained a hypertensve response to ICV clonidine when spread into the fourth ventricle was prevented by externalising the aqueduct between the 3rd and 4th cerebral ventricles. This involved 100 ug of clonidine and the pressor response appeared rapidly. Trolin (1975) compared conscious, anaesthetized and decerebrate rats. In the conscious animals only a brief hypertension appeared, in anaesthetized a pressor then depressor action occurred and in decerebrate only a hypotensive action was noted. A pressor response to clonidine still failed to appear even with 100 ug/Kg IV. Trolin proposes that supracollicular structures act to maintain blood pressure and generate the pressor response to clonidine. His results are incompatible with the widely held view that the brief pressor action of clonidine is a peripheral effect. Shaw et al (1971) noted that the hypotension following clonidine was greater in pontine animals than intact but that the fall in heart rate was reduced, again arguing that the forebrain operates to maintain arterial pressure.

Constantine & McShane (1968), Laubie et al (1976) and Katic et al (1972) administered a small dose of clonidine into the internal carotid artery. This led to no alterations in blood pressure. Therefore no role for the forebrain as a site of action for clonidine is indicated, pressor or depressor. This does not mean that structures rostral to the pons are not involved in the changes in blood pressure seen after clonidine, just that clonidine does not act in those areas.

Schmitt & Schmitt (1969) found the response to clonidine unaltered in decerebrate cats and dogs, even when the decerebration extended to the pons, in addition decerebration during the response to clonidine did not restore blood pressure.

Struyker-Boudier & Van Rossum (1972,1974) and Struyker-Boudier

et al (1974) microinjected clonidine into the rat hypothalmus and produced a prolonged dose dependent fall in arterial pressure and heart rate. The fall in heart rate appeared with 1 ug whilst 3-20 ug progressively lowered blood pressure. Myers (1966) shows that 1 ul, the volume used by Struyker-Boudier & Ván Rossum, microinjected into the hypothalamus is likely to spread no more than 2.2 mm. Further the dose used is ug/animal not ug/Kg equivalent to perhaps 80 ug/Kg. The prolonged response reported suggests that movement away from the site of injection is slow and that in consequence levels of clonidine are maintained. The most remarkable aspect of these two papers is the dose dependent nature of the response. Timmermans et al (1977) and Conway & Jarrott (1980) show that only 2% of the clonidine given IV resides in the brain. Thus even if the 80 ug/Kg was distributed thoughout the brain it is equivalent to 4 000 ug/Kg IV, clearly of no relevance to the normal actions of clonidine. Philippu et a (1979) found that clonidine enhanced the pressor response to stimulation of the posterior hypothalamus and in higher doses reduced the response. Philippu used superfusion to apply clonidine to the hypothalamus and concentrations of between 10^{-3} and 10^{-5}M were employed, they are far in excess of the concentrations likely to follow IV administration.

The evidence for a hypotensive action of clonidine in the forebrain is poor.

Medulla/Pons

Decerebration experiments (Schmitt & Schmitt 1969) and IVert experiments point very strongly to a site of action for clonidine within the medulla. Microinjection and topical administration have been used to further delineate the site.

Pons

Extending decerebrations below the level of the Pons does not abolish the hypotensive action of clonidine. This suggests that the site of action following IV administration of clonidine is likely to be in an area caudal to the pons. Though the lesion may alter the operation of the undamaged areas, by removing the influence or more rostral structures, and hence the alter action of clonidine on these areas.

Ventral Surface Of The Medulla

A chemosensitive zone on the ventral surface of the brainstem is a favoured site for clonidine's hypotensive action (Bousquet & Guertzenstein 1973). After cannulation of the aqueduct between the third and fourth ventricles the hypotensive response to intraventricular clonidine disappears and is replaced with a hypertensive action, suggesting that the hypotensive site is outside the lateral ventricles, cisterna magna and third ventricle, further the site is accessible from the CSF. Topical application on the ventral surface of the brainstem revealed the area S, lateral to the pyramids caudal to the trapezoid body and rostral to the hypoglossal roots. Bilateral but not unilateral application reduces blood pressure. Lesioning abolishes the action of IV clonidine (Bousquet et al 1975) and the ipsilateral hypothalamic pressor response. Alone lesioning leads to a reduction in blood pressure. Microinjection of clonidine produces an immediate fall in blood pressure (Bousquet et al 1979), reduction of the pressor response to electrical stimulation of the posterior hypothalamus and in the fall in blood pressure following stimulation of the anterior hypothalamus. Piperoxan IV reestablishes both pressor and depressor effects of hypothalamic stimulation following intracisternal clonidine.

The area S is associated with respiratory responses to superfusion with solutions of different acidity (Loeschcke & Koepchen 1958). The topical application of a variety of drugs gaba, glycine, pentobarbitone, clonidine, carbachol, dopamine results in a fall in blood pressure (Guertzenstein 1973). Anatomically it is associated with the nucleus paragigantocellularis and nucleus reticularis lateralis

Criticism of this body of work stems from the quantities of clonidine employed. Topical application involved 10 ul of 0.05-1 mg/ml and microinjection 4 ug in 0.5 ul and ICV 100 ug/Kg. The volume of distribution is likely to be restricted and a concentration markedly in excess of that following IV administration undoubtedly achieved. Until this is resolved the area S cannot be considered seriously as the site of action.

Further the onset of action after topical application is rapid yet that consequent on ICV dosing is widely reported to be slow and

progressive suggesting that topical administration does not mimic the response obtained after ICV. However Bousquet & Guertzenstein (1973), the paper initially associating clonidine with the area S, found a rapid fall in blood pressure after ICV administration. A large quanitity of clonidine was employed which may be the key to fast actions on the edge of the ventricular system.

Among other drugs acting on the area S is procaine, a local aneasthetic. Clonidine can, in high concentrations act in this manner, a possible explanation for this body of work. Bilateral cooling of the area S causes respiratory depression and a reduction in blood pressure, which recovers first (Schlafke & See 1980,Wennergren & Oberg 1980). This also argues for a nonspecific action of clonidine at this site. Though Schlafke & See (1980) recording electrical activity below the area S found two populations of neurons, one was responsive to pH alterations and the second to lofexidine, an imidazole presumably with a clonidine like action. The imidazole increased neuronal activity and led to a concomitant reduction in blood pressure, an effect not compatible with the local cooling response. Electrical stimulation elicits a pressor response (Trouth et al 1973), seemingly inconsistent with imidazole mediated stimulation of neurons in the area.

There is evidence for the area S as a site of action for clonidine but doubts, arising from the quantities of drug employed and the presumed limited volume of distribution, exist.

Medullary Reticular Formation

Chen & Chan (1979) and Chan & Koo (1978) found that placing lesions in the medial reticular formation, which in itself did not alter blood pressure, prevented IV clonidine from lowering blood pressure. Further local injection of 0.25 ug/Kg of clonidine caused a prolonged fall in blood pressure. Electrical stimulation of this area lowers blood presssure. Bousquet et al (1931) working with cats bilaterally injected 75 ng/Kg clonidine into the nucleus reticularis lateralis and found a rapid fall in blood pressur. Placing lesions in this area lowers blood pressure whilst electrical stimulation is pressor. The authors relate the microinjection to areas that may be reached after topical application on the area S.

The iontophoretic study of Anderson & Stone (1977) is

consistent with an action within the medullary reticular formation.

Floor Of The Fourth Ventricle

Schmitt & Schmitt (1969), Srimal et a (1977) and Dhawan et al (1975) all obtained reductions in heart rate and blood pressure with topical application of clondine on the floor of the fourth ventricle. Concentrations of 0.1-1.0% were employed and the corresponding concentration achieved in the underlying tissue are unknown but are likely to be high. The area contains alpha 1 adrenoceptors (Young & Kuhar 1980) which provide a mechanism of action but not for low concentrations.

Nucleus Of The Solitary Tract

Antonaccio & Halley (1977) damaged the NTS which prevents reflex bradycardia, the nucleus receives the baroreceptor inputs, but did not prevent clonidine lowering blood pressure. Though it seems that the enhancement of the baroreflex arc by clonidine requires the integrity of this area. It is not fully established that clonidine acts directly at this site.

Spinal Action

Clonidine has no hypotensive action after spinalization. In tetraplegics bradycardia, reduced salivation and sedation follow oral administration (Reid et al 1977). The bradycardia was probably vagally mediated and the absence of blood pressure changes not due to altered drug kinetics (Reid et al 1977). In cord transected dogs (Ganong et al 1978) clonidine 30 ug/Kg IV was only pressor, in spinal cats 10 ug/Kg IV similarly (Constantine & McShane 1968) or 30 ug\Kg IV (Schmitt & Schmitt 1969) produced no fall in blood pressure. After transection blood pressure falls precipitously then slowly recovers to the original level but even after recovery clonidine is only pressor (Tangri et al 1977) (Petty et al 1976), 30 ug/Kg IV led to a prolonged and enhanced rise in pressure.

In spinal animals sympathetic tone is low and the influence of descending systems removed. In consequence an action on descending tracts, spinal interneurons or sympathetic efferents would not be apparent. When sympathetic tone is stimulated a hypotensive action in spinalized animals may be seen. Haeusler (1976) used strychnine,

1 mg/Kg IV, or stimulated sympathoexcitatory tracts in the dorsal lateral funiculus and showed inhibition of the evoked sympathetic activity by clonidine 3-30 ug/Kg IV or alpha methyl dopa 100 mg/Kg IV. Dhawan et al (1975) used spinal compression to elicit a pressor response in spinalized cats which was attenuated by clonidine 1-2 ug/Kg IT. Koss (1976) found the centrally or peripherally evoked electrodermal response reduced by clonidine. Sinha et al (1973) evoked splanchnic nerve discharges by stimulating the central end of sciatic nerve and found the response reduced by clonidine. Baum & Shropshire (1977) noted a reduction by clonidine of the sympathetic nerve activity evoked by stimulation of descending tracts in the spinal cord or afferent nerve stimulation. Franz et al (1978) found clonidine reduced the evoked response in preganglionic sympathetic nerves from either somatic afferent or intraspinal tracts.

In cord intact anaesthetized rats IT clonidine 0.4-10 ug/Kg has a hypotensive action (Finch et al 1979, Connor & Finch 1981). The onset of action is faster after IT than ICV administration suggesting a spinal site of action (Dhawan et al 1975). Lo Pachin & Rudy (1981) found a reduction in nerve activity in the lumbar sympathetic chain after IT clonidine. 6.8 ug/rat was employed and the peak action appeared forty minutes after administration.

Walland (1978) questions the importance of the spinal site. A reduction in the pressor response evoked by tibial nerve stimulation with high voltage and high frequency followed IV clonidine, oxymetazoline or tramazoline. IVert, ICV and intralumbar clonidine failed to alter the evoked response and as the two other agents employed do not cross the blood brain barrier an arguement is developed for a site of action outside the CNS.

Clearly clonidine can show a hypotensive action within the spinal cord but this may not be involved in the responses following IV dosing. The technique for IT administration (Yaksh 1976) results in good localization with limited spread along the cord from the tip of the cannula. The spread of the effective dose 0.4-10 ug/Kg is not known but is likely to be small and therefore may reach concentrations in excess of those found after IV administration. A few ug/Kg IV show a hypotensive effect and the dose is distributed throughout the whole animal. In view of the delayed appearance of the peak effect (La Pachin & Rudy 1980) and large dose adminstered a

spinal action may not be invovled. The delay may reflect the time taken to escape from a depot created in the spinal cord. The somatic sympathetic response, seen with stimulation of the cut ends of afferent nerves, has two components fast and slow (Sato & Schubert 1973), Coote & Downman 1966). The first involves only the spinal cord while the second requires supraspinal structures. Their susceptibility to clonidine differs, in low doses clonidine is more effective in reducing the late component (Baum & Shropshire 1977, Sinha et al 1973). Conversely, higher doses are required to reduce the spinal component of the reflex, the response evoked by intraspinal stimulation and the pressor responsse to hypothalamic stimulation (Sinha et al 1973). In other studies, where discrimination between medullary and spinal actions could not be made, clonidine was active at a spinal level in low doses (Franz at al 1978, Haeusler 1976).

Clonidine appears to act postsynaptically, acting after reserpinization (Franz et al 1978), IT 6OHDA and IT 5,6DHT (Connor & Finch 1981). The latter two drugs damage catecholamine and indolamine containing neurons. Clonidine is effective on the spinal pathway which does not contain catecholamines and presumably acts on the preganglionic sympathetic cell body.

The action of clonidine is antagonized by IV alpha antagonists intimating that the effect is mediated in this manner (Franz et al 1978, Haeusler 1976, Sinha et al 1973, Finch et al 1979). Finch found antagonism of the hypotensive action by prazosin, an alpha 1 adrenoceptor antagonist, but no action with phenylephrine. Further a range of alpha 2 adrenoceptor antagonists given IT lowered blood pressure. This inconsistent with the idea of alpha receptors having an inhibitory action on the sympathetic nervous system at the spinal level. These results may reflect problems with the IT technique. Franz et al (1978) proposes an action on 5HT receptors. This dichotomy revolves around the action of descending tracts containing noradrenaline and 5HT on preganglionic sympathetic neurons.

The consensus is that descending indolamine systems are inhibitory (Coote & Maclean 1974, Franz et al 1978, Neumayr et al 1974, Hare et al 1972). Two positions exist on the role of noradrenergic systems that they are inhibitory (Coote & Macleod 1974, Haeusler 1976) or that they are excitatory (Neumayr et al

1974, Hare et al 1972, Taylor & Brody 1976). Iontophoretic studies show an inhibitory role for noradrenaline on spinal preganglionic sympathetic neurons (De Groat & Ryall 1967, Hongo & Ryall 1966, Coote et al 1979). Blessing and Reis (1982) produced a hypotensive response by stimulating, electrically and with L-glutamate, the A1 area in the ventrolateral medulla. This area contains catecholamines and the stimulation experiments support the idea of descending inhibition involving this type of neuron. The opposing view derives from biochemical studies using drugs that may not have a single action. The iontophoretic studies with 5HT show it to be excitatory and in consequence the existence of an interneuron is postulated (Coote et al 1979).

How important a spinal action is, in the hypotensive response to clonidine is not clear. It is suggested that high doses are required to reduce sympathetic tone through this site and the concentrations resulting from IT administration are likely to be excessive.

Conclusions

Despite a large body of work the origin of the hypotensive effect of clonidine it is not unequivocably established. It is clear that the acute response involves areas within the brain and spinal cord and in all probability involves alpha 2 adrenoceptors. However there is also evidence for an action outside the CNS on baroreceptors, presynaptic receptors and vascular smooth muscle senstivity.

A large body of work exists on the site of action but problems with drug concentration and the multiple pharmacological actions of clonidine surround each proposed site. The question is not whether clonidine can act at a given site but if the site is involved in the clinical response

The search for a unitary site of action may be misconceived and the cardiovascular actions of clonidine result from the summation of minor effects in a multiplicity of areas. Sufficient uncertainty exists to justify yet more research in this field.

Anaesthetics Different Hypertensive Models And Clonidine

The absence of a hypotensive effect of clonidine has been

reported in conscious rats (Trolin 1975, Pals 1975, Zandberg 1977, Henning et al 1975). In addition the brief hypertensive episode associated with bolus IV clonidine administration was attenuated in barbiturate anaesthetized rats and could not be demonstrated in decerebrate animals even with large doses (Trolin 1975, Henning et al 1975). The authors suggest that supracollicular structures are involved in the hypertensive action of clonidine . Their role is likely to be permissive rather than as a site of action. A pressor action mediated through these areas would require the hypertension to involve increased sympathetic nerve activation, which has not been reported during the hypertensive interlude, and that IC administration should lead to hypertension, again not reported. Further work on isolated tissues and pithed rats indicates that the hypertension is peripherally generated. The role of the supracollicular structures then must be assumed to be permissive, allowing hypertension to appear by inhibiting the compensatory mechanisms. This is also hard to accept as it requires that in the anaesthetized and decerebrate animal the functioning of the baroreflex arc is greatly enhanced, to such an extent that the pressor action is overwhelmed. The baroreflex operates as as a negative feedback loop and as such cannot operate to completely eliminate the imposed stimulus, a load error should appear.

Anaesthetic can enhance the operation of the baroreflex arc, decreasing the response to a pressor stimulus. Brezenoff (1973) found that high levels of pentobarbitone reduced the effect of IV noradrenaline on arterial blood pressure whilst lighter anaesthesia increased the magnitude ot the response. A range of anaesthetics did not have the a similar effect on the pressor response to noradrenaline and may involve both central effects and alterations in vascular responsiveness.

Pals (1975) found even large doses of clonidine ineffective in in conscious rats but sodium depletion, with frusemide, which did not alter arterial pressure made clonidine hypotensive.

Clonidine appears to cause catecholamine release from the adrenal gland (Zandberg 1975) having a greater hypotensive action after adrenalectomy in conscious and urethane anaesthetized rats. only a slight enhancement appeared in pentobarbitone treated animals. It is unclear whether a centrally-mediated or direct action

is involved as denervation of the adrenals was not undertaken and no reports on the actions on adrenal sympathetic efferents appear to exist.

A differential effect on urethane and pentobarbitone anaesthetized rats was not noted (Bousquet et al 1977) in whose hands a reduction in arterial pressure was seen with these two anaesthetics but not with ketamine, althesin or chloralose. In the Bousquet et al study clonidine led to a prolonged hypertension with althesin, work at ICI does not support this finding and instead a reduction in arterial pressure appeared (P. Marshall personnal communication). Bolme et al (1974) were able to obtain a fall in arterial pressure in the chloralose anaesthetized rat again contradicting Bousquet et al (1977). In the rat Timmermans et al (1977) found greater falls in arterial pressure when using barbiturate anaesthetized than ether treated rats although no pharmacokinetic changes were apparent.

Despite a faiure of a number of authors to find a reduction in blood pressure in conscious rats it has been noticed (Meyer 1977, Salzman 1979, Finch & Hicks 1980, Christersson et al 1979).

The effectiveness of clonidine varies in different hypertensive models (Dadkar et al 1979). The action was greatest in SHRs and smaller in renal and Doca/salt rats. At the end of the first day of treatment the reduction in blood pressure was similar in all three groups of rats. The new level of pressure was maintained in the renal and Doca/salt animals but in the SHRs arterial pressure fell further over the next few days. Differences in vascular reactivity to clonidine are involved (Dadkar et al 1979). Plasma renin falls with clonidine in animals and man and the pretreatment plasma concentration is related to the subsequent reduction in arterial pressure. Pals (1975) only found clonidine effective in conscius rats after sodium depletion, this increases plasma renin activity which falls after clonidine.

Overall clonidine can lower arterial pressure in conscious and anaesthetized rats but the anaesthetic and type of rat used are important. The failure of some authors to find a hypotensive response in conscious rats suggests that the use of anaesthetized animals is preferable. The reported differences between anaesthetics and the failure of clonidine to interact with the adrenals in

barbiturate treated animals suggest that this is the simplest preparation in which to investigate the hypotensive properties of clonidine. The diverse results reported with different anaesthetics and the work on decerebration deserve further attention.

General Methods

The hypotensive and bradycardic action of clonidine is readily demonstrated in anaesthetized rats which were used in all experiments. Rats of both sexes within a weight range of 140-350 gms were used.

Initially urethane was used to provide anaesthesia but as the CASE award involved working at ICI Alderley Park where this substance is deemed carcinogenic a switch was made to Inactin. Inactin (thiobutobarbitone manufactured by BYK) is a barbiturate anaesthetic that provides several hours of stable anaesthesia after a single dose. Initially 100mg/Kg IP was given from a freshly made up stock solution of 50mg/ml in water and the dose increased progressively at 15 minute intervals until an acceptable level was achieved, usually 115-135mg/Kg sufficed. Lower doses are reported in the literature but those used are in the range used at ICI.

Blood pressure was measured using standard Lectromed/CEC strain gauge transducers connected to either the carotid artery or femoral vein. Static calibration was made using mercury manometers and dynamic calibration was not undertaken as suitable equipment was not available and a detailed knowledge of the waveform was unnecessary for the experimates undertaken. Except where mentioned the femoral artery was used in preference to the carotid as to use the latter results in one set of baroreceptors operating at a pressure below arterial pressure. This was shown in a number of animals by cannulating both the central and peripheral end of a carotid artery. Although changes in arterial pressure are followed by the centrally located cannula the pressure recorded is always lower than that recorded at the cardiac end of the carotid. This suggests that the relevant baroreceptors and chemoreceptors are likely to be operating differently from those on the contralateral side and femoral arterial cannula were used to obviated this problem.

Cannulae were made from polythene tubing PP50 with the tips stepped down to a size just sufficient to enable introduction into blood vessels.

Heart rates were obtained from the arterial trace using ratemeters triggered from the pressure pulse. Two ratemeters were used, a Devices instantaneous ratemeter used over a 0-500 min-1

range and a ratemeter built at ICI that required several beats to adjust to a change in heart rate, this machine could be offset allowing a 200 beat interval to provide full scale deflection on the pen recorders.

A variety of Devices/Lectromed hot wire recorders were used to obtain a record of the experiment.

Once anaesthetized the animals had a rectal probe inserted to record temperature. Using either a heating lamp or blanket controller, of local design, rectal temperature was maintained within 36.5-37.5 C.

IV administration was achieved by drawing the drug into a long polythene cannula which was then connected to a venous cannula. The syringe was replaced with one containing saline and drug flushed out of the cannula with 0.2 ml of saline. A microlitre syringe was used to draw up drugs which permitted the use of small injection volumes. This was considered important as repeated drug injections could otherwise involve large cumulative volumes of saline.

Intravenous Clonidine And The Inactin Anaesthetized Rat

Clonidine lowers blood pressure and heart rate in anaesthetized animals and as these animals are more amenable to experimental manipulation they were used throughout the study.

The initial series of experiments were undertaken to establish the nature of the clonidine-induced response in the inactin anaesthetized rat.

Experiments

1) The effect of a carotid arterial cannula on carotid pressure.
2) IV clonidine administration.
3) Control of resting heart rate.
4) The pathway for clonidine induced bradycardia.
5) Alterations in peripheral resistance after clonidine.
6) Sympathetic efferent activity in the anaesthetized rat.
7) Operation of the baroreflex arc.

Methods

Unless otherwise stated animals were prepared as outlined in the general methods section.

1) One carotid artery was cannulated twice. One cannula was inserted in the directiion of the heart and and second iin the diirection of the head.

3) To remove vagal control of heart function 1 mg\Kg IV atropine was employed. Sympathetic control was curtailed with 1 mg/Kg IV atenolol, a cardioselective beta blocker. Hexamethonium 30 mg/Kg IV was employed to remove neural control of the heart and vasculature. An infusion at 20 ul/min of 90 mg/ml lasting several minutes was used to administer heaxamethonium. Despite this slow method of injection four of the twelve rats given hexamethonium died during or just after administration. The quantity of hexamethonium used was established in a number of preliminary trials where cumulative injections were made until no further reduction in heart rate of blood pressure could be obtained.

5) Peripheral resistance was measured using the pressure generated downstream of a roller pump operating in the constant rate mode. Perfusions were made of the hindquarters and of a hindlimb. The method of Brody et al (1963) was employed for the hindquarters perfusion. This involves double cannulation of the descending aorta. Hindlimb perfusions were established as described in the next chapter but without the large extracorporeal circuit. Flow rates of between 1 and 2.5 ml/min^{-1} were employed for the hindliimb perfusions.

6) The renal and superior cervical nerves were dissected for multiunit efferent recording. The renal nerve was approached with a midline ventral incision, tissues above the nerve were retracted and the peritoneal cavity used to hold the paraffin pool. The superior cervical nerve was approached from the ventral surface of the neck and the skin of the neck arranged to retain the liquid parrafin pool. A tracheal cannula was inserted through the mouth to avoid cutting the trachea which could have acted as a drain for the mineral oil bath.

Recordings were made using bipolar silver electrodes. The signal was passed through a physiological amplifier with the filters set to exclude frequencies above 10kHz and below 80Hz. Tektronix RM 122, Tektronix 3160 and a locally built amplifier were used. The resulting signal was displayed on a Tektronix 5000 series cathode ray osciilloscope (CRO) and a Telequipment DM 64 CRO. A numerical count of action potentials was obtained from a spike discriminator and counter (see appendix 2). Facilities for obtaining taped records of the nerve actiivity were not availible.

7) The baroreflex was studied by perfusing the carotid artery with blood at different pressures, looking for alterations in heart rate and artrial pressure, measured from the femoral artery. The vagi were cut in the neck to remove the influence of cardiac and pulmonary receptors and aortic arch receptors.

The roller pump and feedback loop, described in appendix 1, were used to perfuse the rostral end of the common carotid artery.

Blood for the perfusion was obtained from the caudal portion of the same common carotid. Once the double cannulation of the carotid had been established the other carotid artery was ligated.

Results

1) The pressure recorded from the cranial end of the carotid artery was always much lower than that from the cardiac side. In three rats the arterial pressures were:

Systolic	Diastolic	Mean	Cranial Carotid mmHg
135	100	112	44
145	104	118	51
120	106	111	24

The cranial pressure was not pulsatile and followed that recorded from the cardiac cannula during changes in arterial pressure.

2) Clonidine produced a dose dependent reduction in both heart rate and mean arterial pressure. Fig 2.1 a,b,c and tables 2T3-8. The reduction in heart rate and blood pressure were prolonged. A brief rise in blood pressure follows bolus IV clonidine, this is not shown in Fig 2.1 a,b as it did not extend to the first point on the graph, 1 min post injection. After hexamethonium pretreatment the magnitude and duration of the pressor effect were enhanced, from a peak rise in systolic pressure of 31 mmHg and a duration of less than one minute to a mean rise of 64 mmHg and a duration in excess of three minutes Fig. 2.2.

3) Control of resting heart rate.

Atropine 1 mg/Kg IV did not alter heart rate or blood pressure, Fig 2.3 a, table 2T.1.

Atenolol 1 mg/Kg IV reduced heart rate but not mean arterial pressure, Fig 2.3 a,b, table 2T.1. In five of the six rats given atenolol the heart rate was more stable after treatment than before, in the sixth rat heart rate was stable at all times. Stability refers to variations about the mean.

Heart rate was reduced by injections of pressor drugs, infusion of pressor drugs or alteration in blood pressure around the

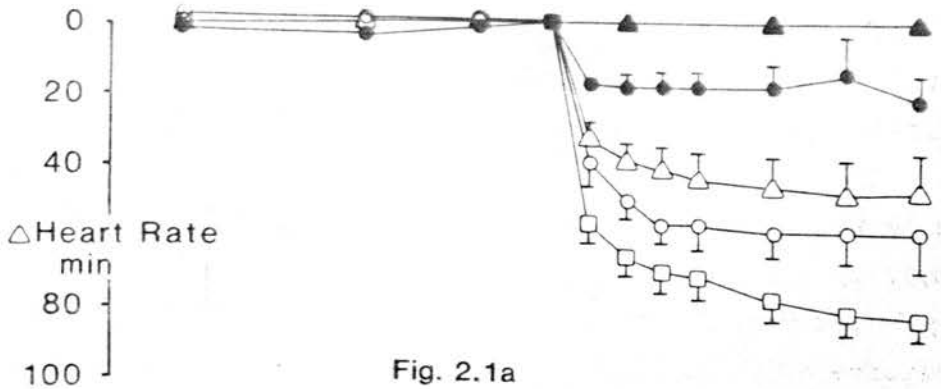
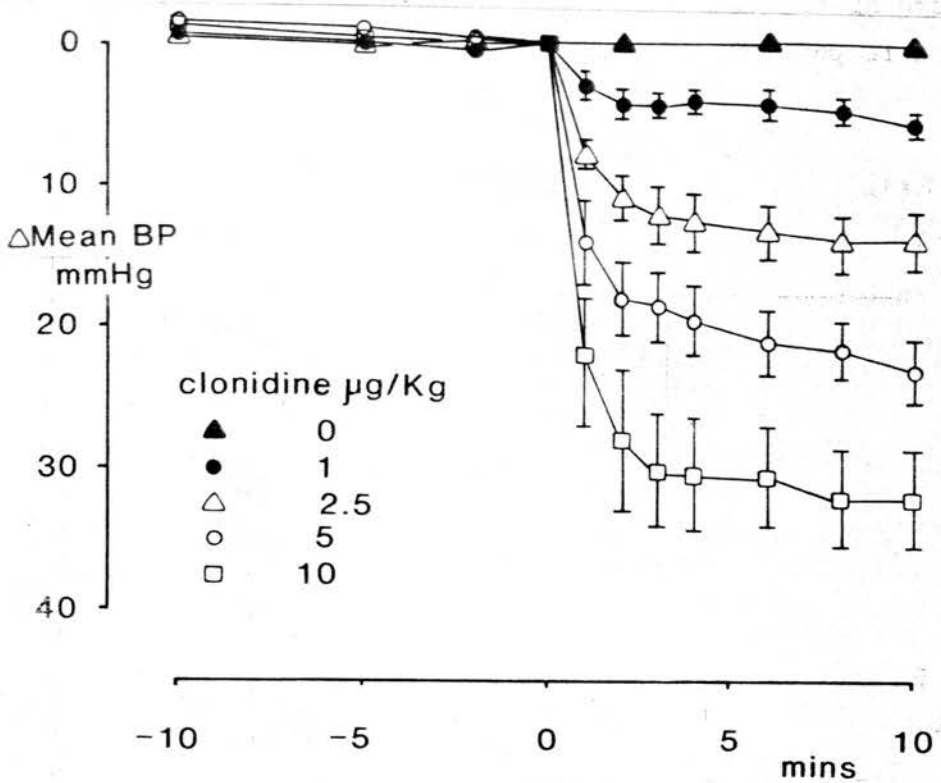


Fig. 2.1a



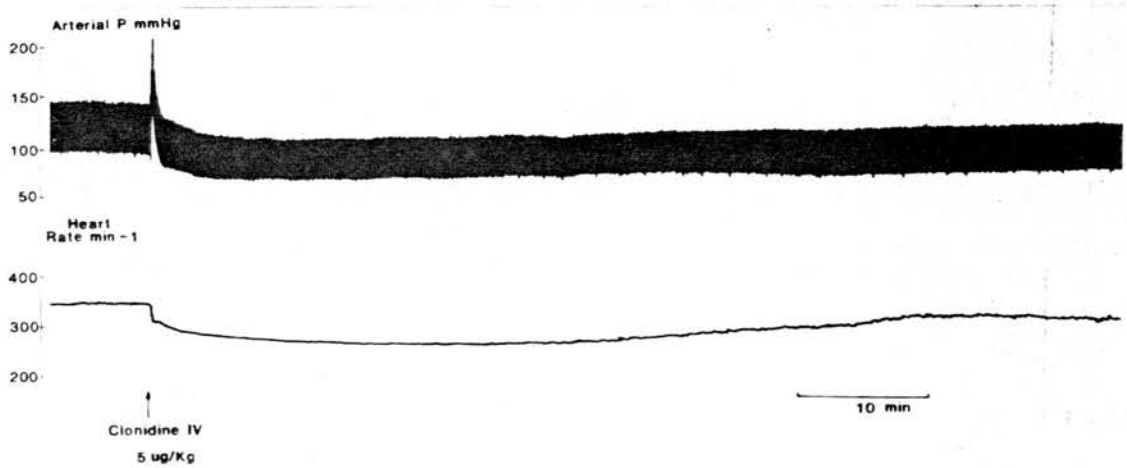


Fig. 2.1b

Fig. 2.1a,b,c

IV clonidine given to an Inactin anaesthetized rat.

Note initial pressor response, subsequent fall in blood pressure and bradycardia.

a) Acute response to a bolus IV injection. Readings taken at -10,-5,-2,0,1,2,3,4,6,8 and 10 mins after injection.

1ug/Kg N=6, 2.5 ug/Kg N=11, 5 ug/Kg N=14, 10 ug/Kg N=12

d) Response of one animal to 5 ug/Kg. Note brief rise in arterial pressure after bolus injection.

c) The response to 10 ug/Kg followed for 55 mins. N=12

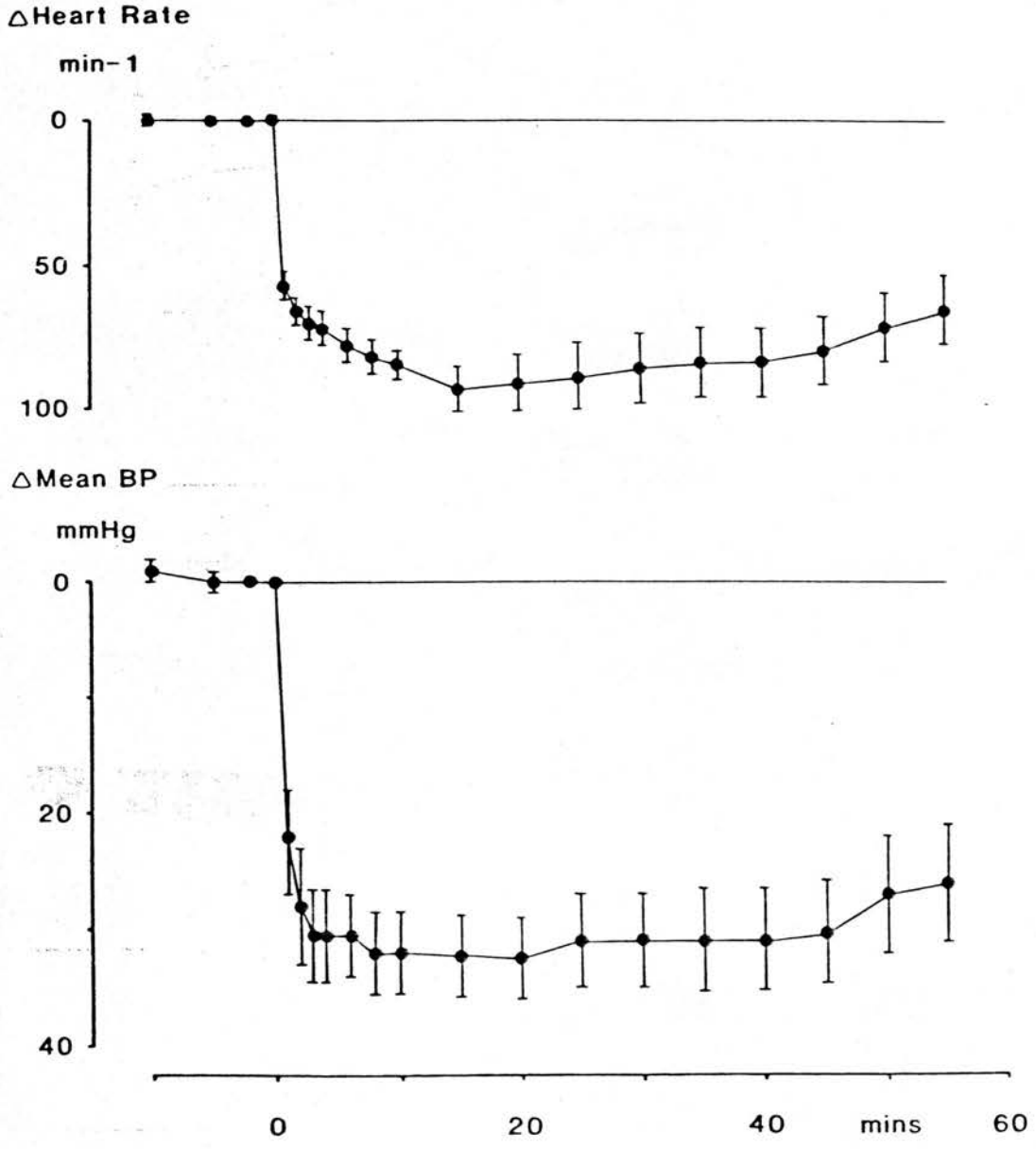


Fig. 2.1c

10 µg/Kg clonidine i.v.

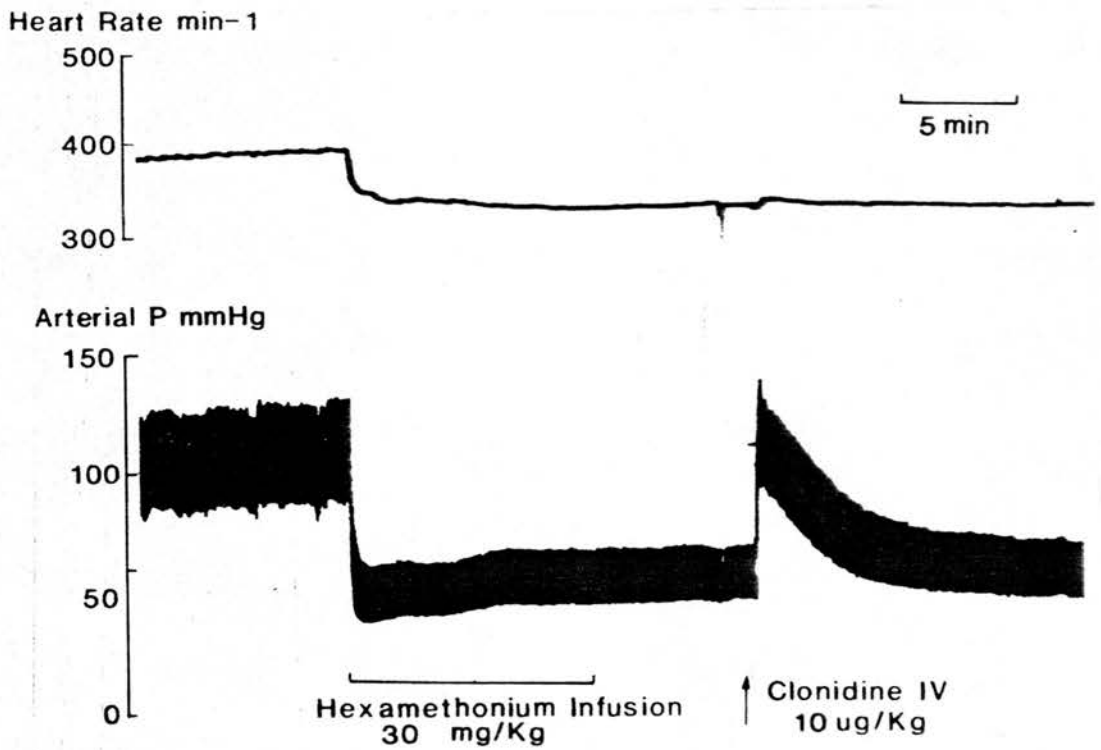


Fig. 2.2

IV Clonidine After Hexamethonium

Note persistance of pressor response, compare with Fig. 2.1c

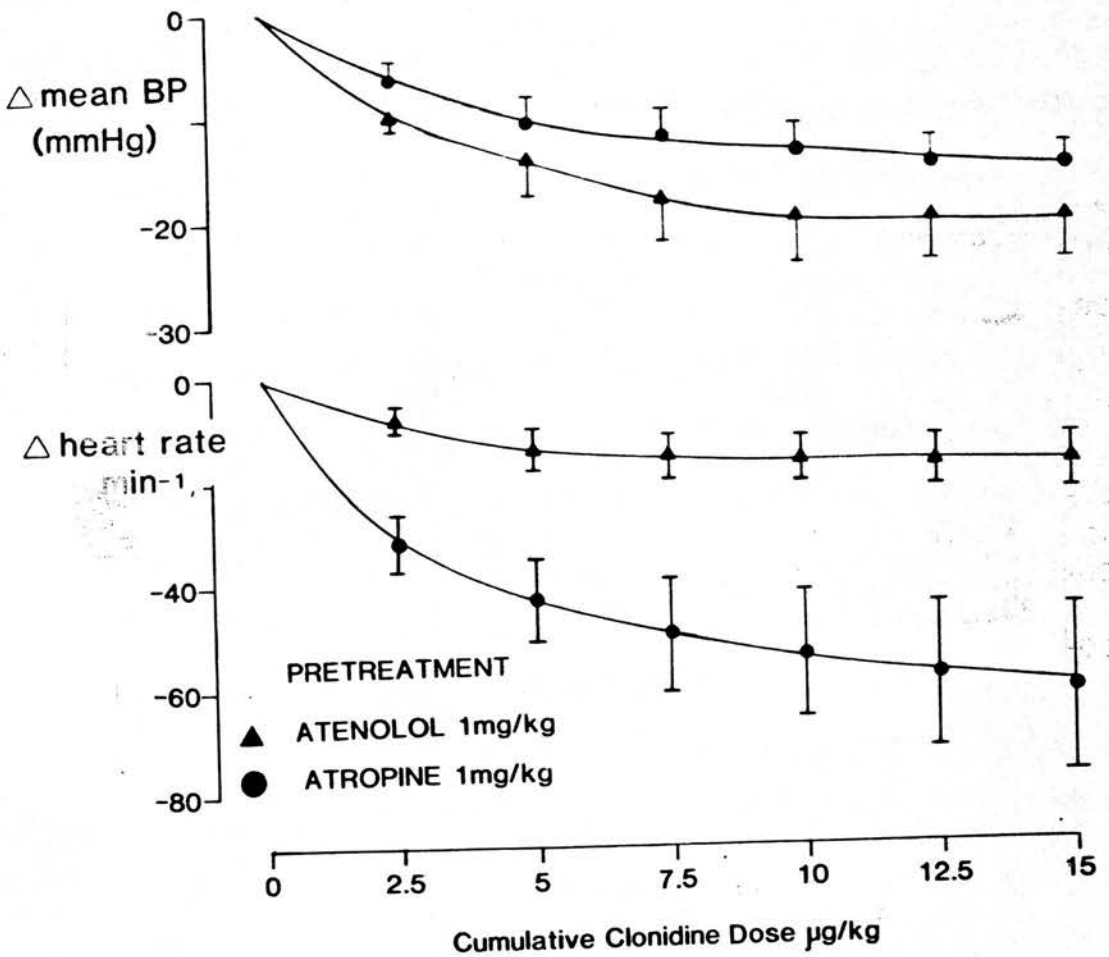

0
△mean BP
(mmHg)
-20
-30
0
△heart rate
min-1
-40
-60
-80
PRETREATMENT
ATENOLOL 1mg/kg
ATROPINE 1mg/kg
0
2.5
5
7.5
10
12.5
15
Cumulative Clonidine Dose µg/kg

Fig. 2.3a

Effect Of IV Atropine And Atenolol On Blood Pressure And Heart Rate.

Administration IV bolus.

Atropine 1 mg/Kg N=8, Atenolol 1 mg/Kg N=6.

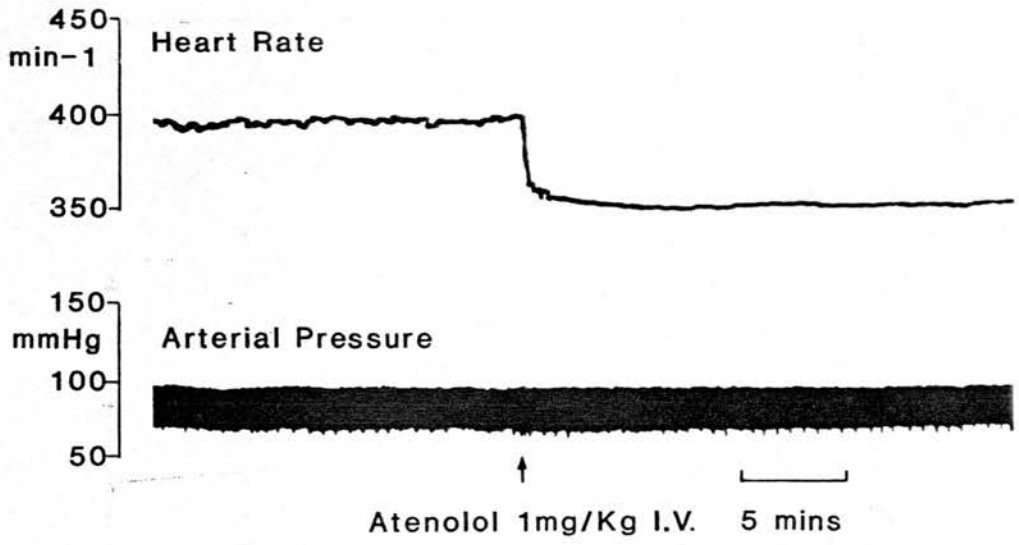


Fig. 2.3b

Response To IV Atenolol 1 mg/Kg IV

Note fall in heart rate but not blood pressure and the stability of heart rate after atenolol.

baroreceptors (described in Appendix 1) Fig. 2.6b, 2.7, 3.1a, 3.1b, 3.3c & A1.6.

Hexamethonium, 30 mg/Kg IV, caused a mean reduction in heart rate of 95 beats min^{-1}, reducing heart rate to 354, mean of 8. Subsequent treatment with clonidine only reduced heart rate in one of four rats and then by only 10 beats min^{-1} (Fig. 2.2.).

4) After pretreatment with atropine, clonidine still reduced heart rate and blood pressure, Fig 2.4 a,b and table 2T.2. Pretreatment with atenolol reduced heart rate and greatly attenuated the action of clonidine on heart rate, Fig 2.4 a,b. Cumulative doses of clonidine eventually do not lower heart rate further but a reduction occurs during the brief rise in blood pressure following a bolus injection, Fig 2.4 c,d,e

After treatment with clonidine and atenolol the injection of atropine does not alter heart rate, similarly atenolol after clonidine and atropine has no effect.

5) In two perfused hindquarters preparations clonidine reduced peripheral resistance and heart rate. The preparation proved awkward to establish and further work was discontinued in favour of the perfused hindlimb, Fig 2.5 a,b,c show results from individual experiments. Cumulative doses of clonidine were used, given every seven minutes. Peripheral resistance, arterial pressure and heart rate initially decline but as the dose is increased perfusion pressure and arterial pressure increase though heart rate does not.

6) Sympathetic efferent nerve activity in the inactin anaesthetized rat responds to a variety of stimuli, Fig 2.6. Raising arterial pressure with either injection or infusion of phenylephrine reduced efferent activity. Asphyxia raised nerve activity, arterial pressure and heart rate. Acetylcholine lowered heart rate and arterial pressure and raised sympathetic efferent activity. The traces shown are typical results.

7) The operation of the baroreflex arc can be seen in Fig. A1 6.b (in appendix 1) and fig. 2.7. The latter shows alterations in heart rate and arterial pressure in response to alterations in the

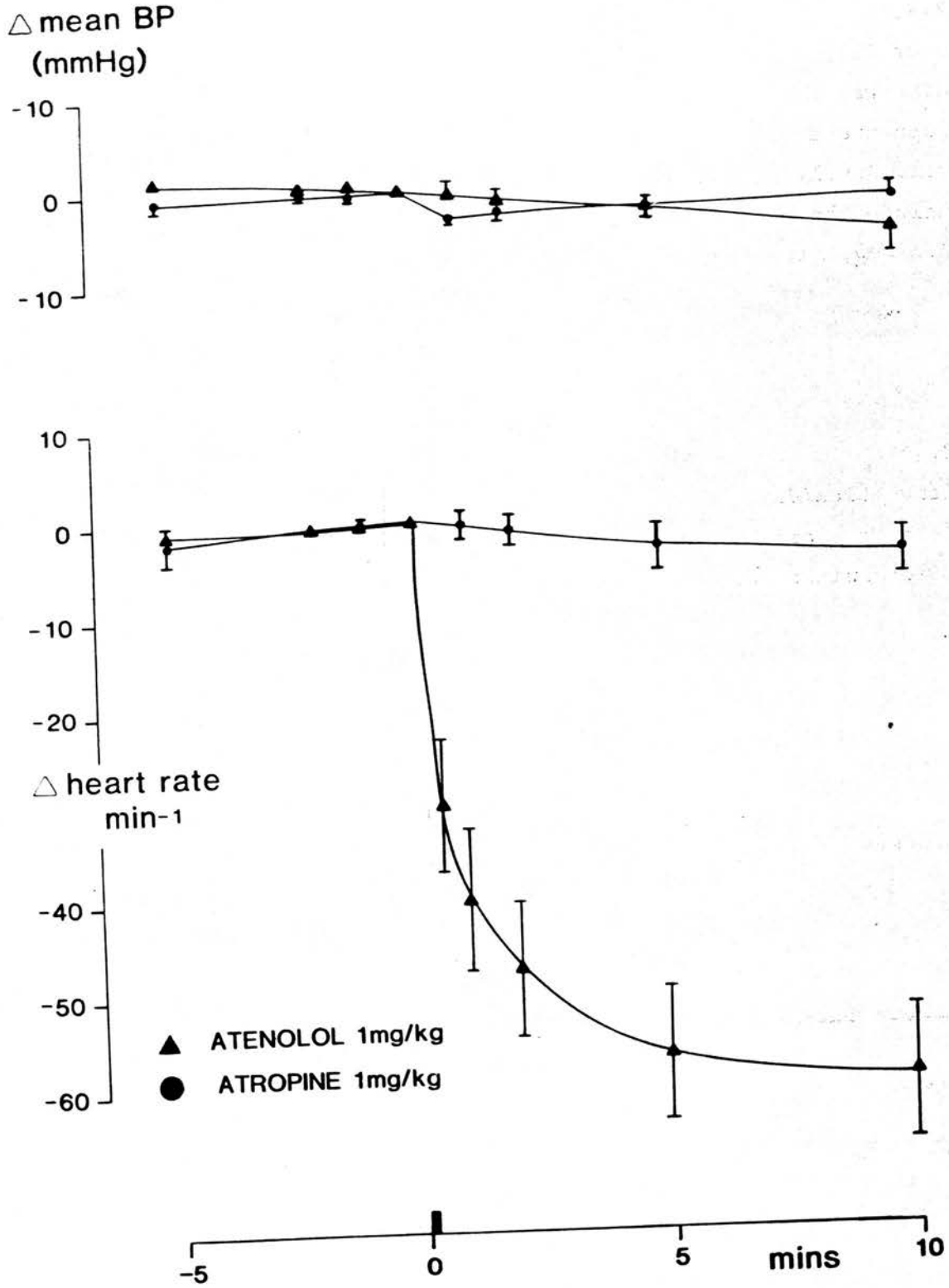

△mean BP
(mmHg)
-10
0
-10
10
0
-10
-20
△heart rate
min-1
-40
-50
-60
ATENOLOL 1mg/kg
ATROPINE 1mg/kg
-5
0
5
mins
10

Fig. 2.4a

Action Of Clonidine After Pretreatment With Atenolol Or Atropine

Clonidine was given cumulatively every 7 mins. The responses used in the graph are those 6.5 mins after administration, this was employed to avoid involving the response to the brief hypertensive episode and include the full response to clonidine.

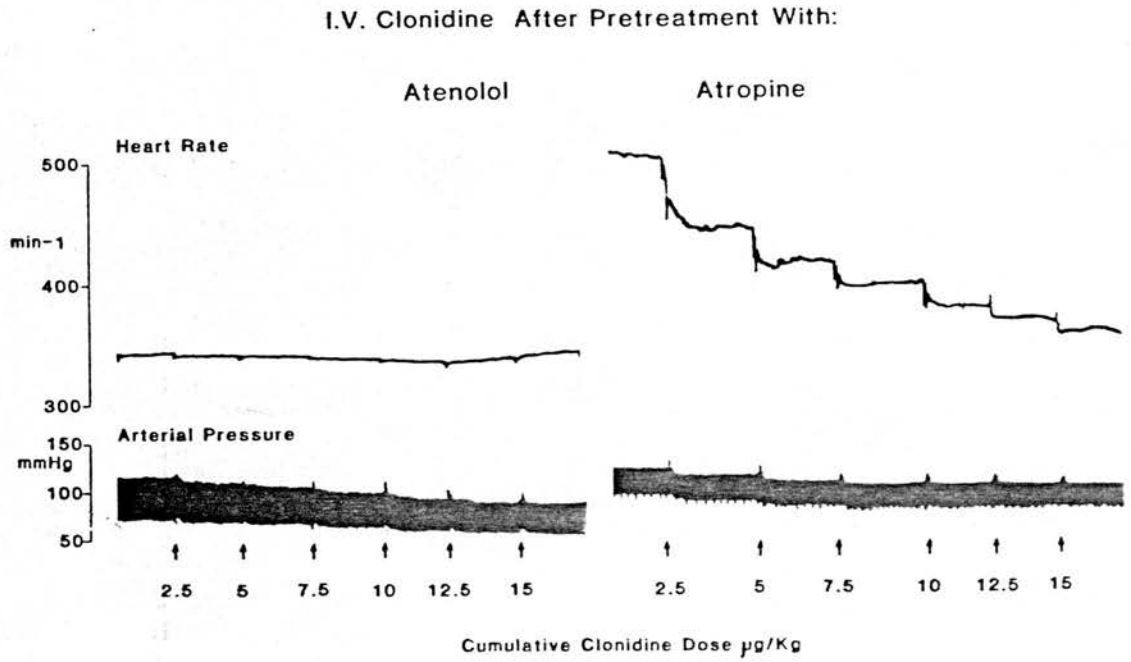
Atenolol N=6, Atropine N=8

Fig. 2.4b

Cumulative Clonidine After Atenolol or Atropine

Effects on two animals.

Note lower initial heart rate in the atenolol pretreated animal.



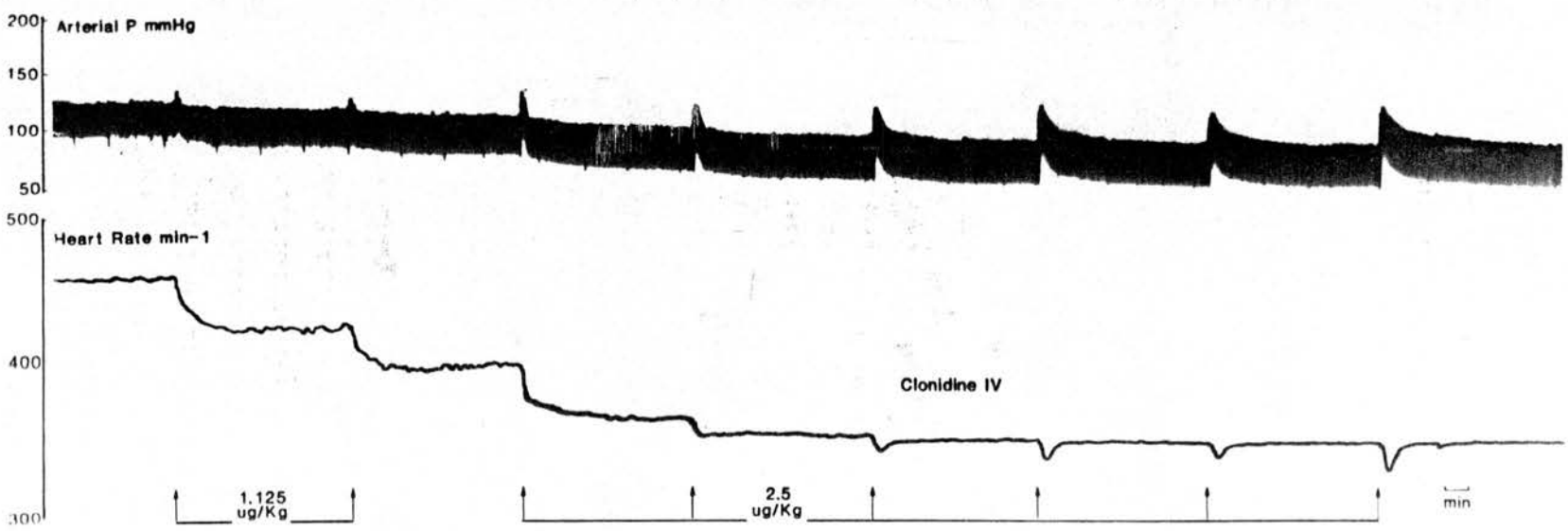


Fig. 2.4c

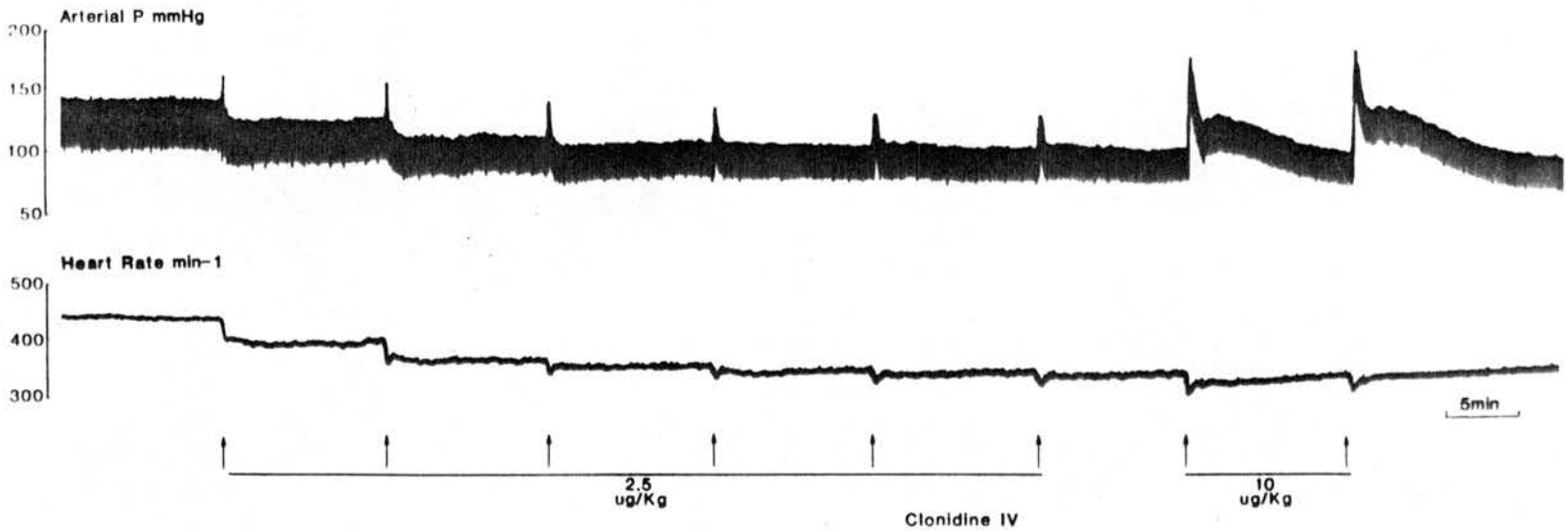


Fig. 2.4d

Fig. 2.4c,d,e

Heart Rate Response To Clonidine During The Pressor Episode

Fig. 2.4 c,d

Cumulative clonidine in two rats, dosed every 10 mins.

Note reduction in heart rate during pressor episode and prolonged hypertensive period after the two last applications of clonidine in Fig. 2.4d.

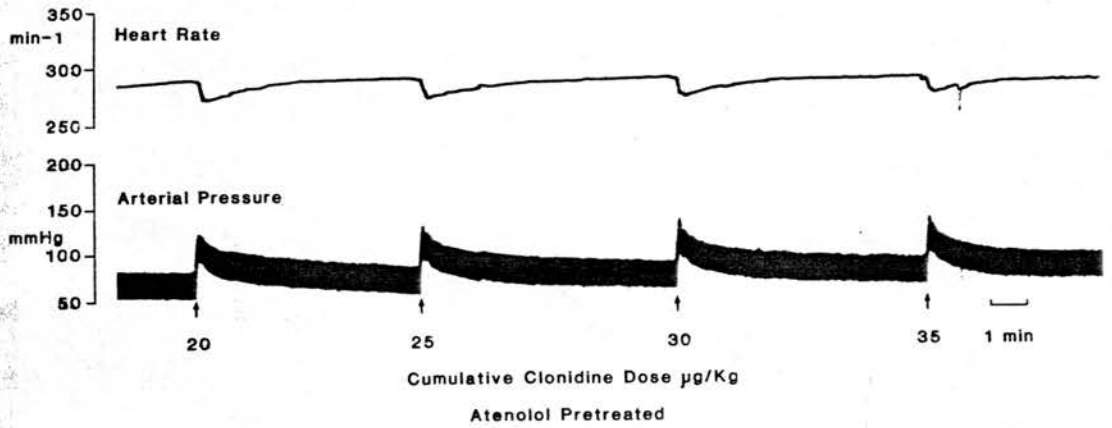


Fig. 2.4d

Heart rate response in atenolol pretreated animal

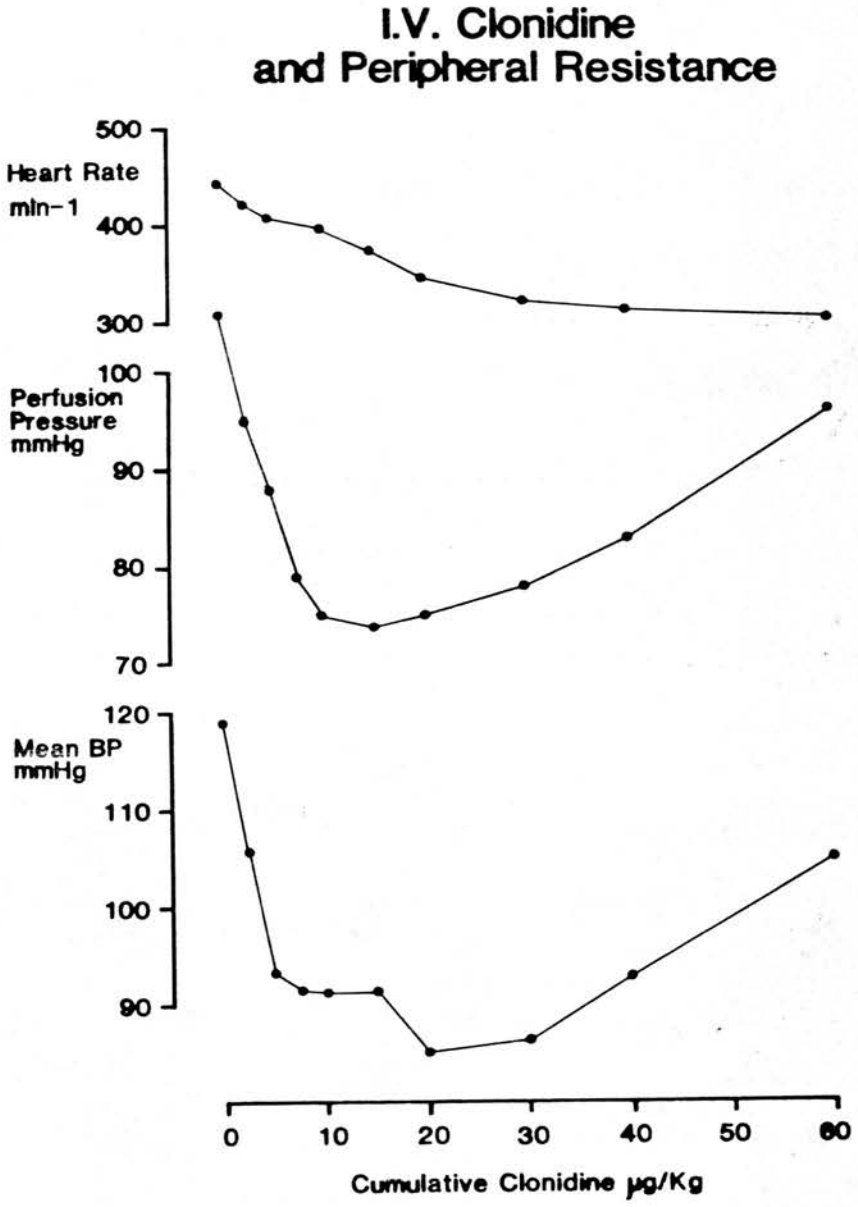

I.V. Clonidine
and Peripheral Resistance
500
Heart Rate
min-1
400
300
100
Perfusion
Pressure
mmHg
90
80
70
120
Mean BP
mmHg
110
100
90
0
10
20
30
40
50
60
Cumulative Clonidine µg/Kg

Fig. 2.5a

Fig. 2.5a,b

Cumulative Clonidine And Peripheral Resistance

Peripheral resistance measured with a perfused hindlimb. Perfusion at constant flow.

Clonidine given every 10 minutes.

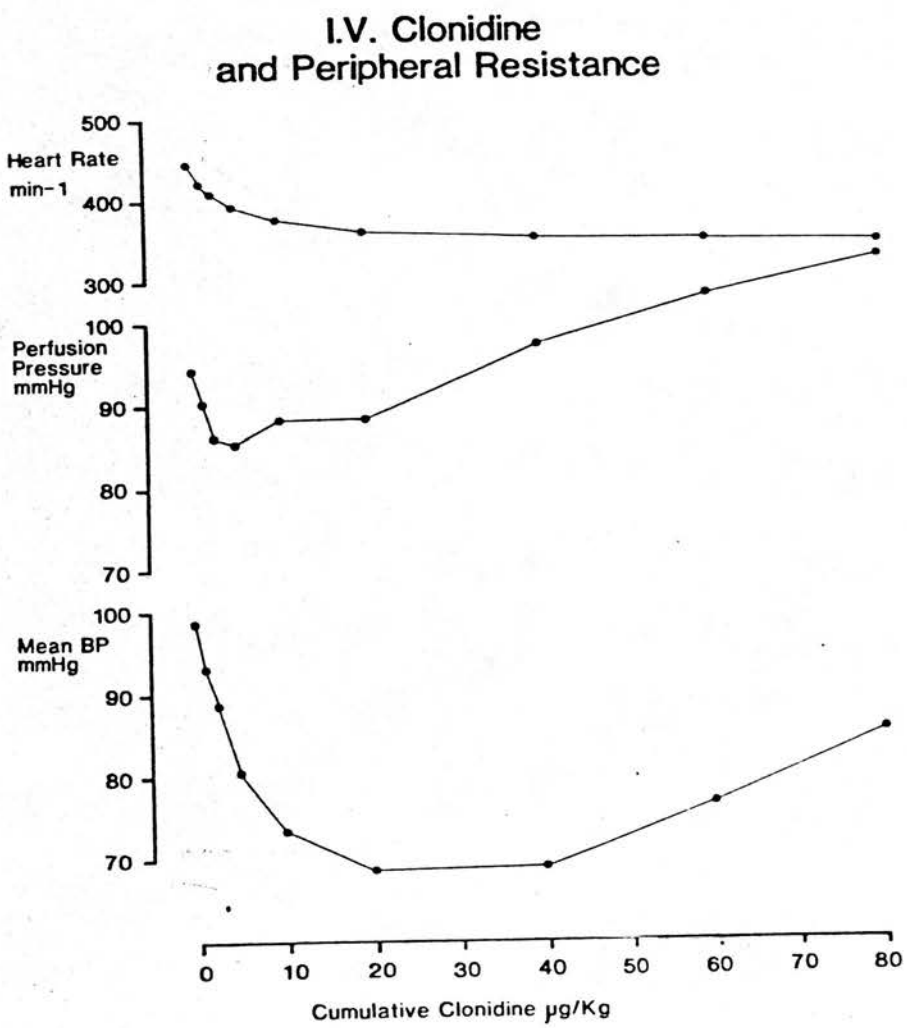


Fig. 2.5b

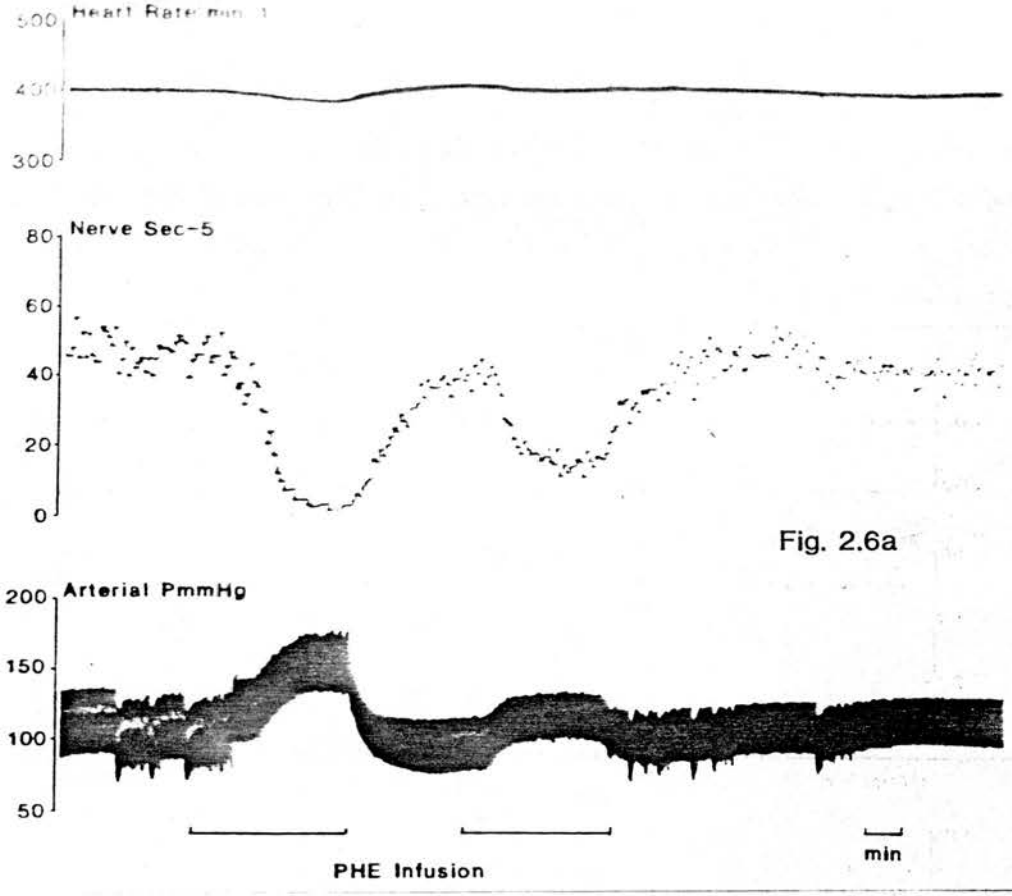


Fig. 2.6a

a) Phenylephrine infusion at two different rates. The reduction in nerve activity follows the onset and magnitude of the pressor effect. Recovery is to the initial resting level of activity. A slight fall in heart rate also occurs. Recording from renal nerve.

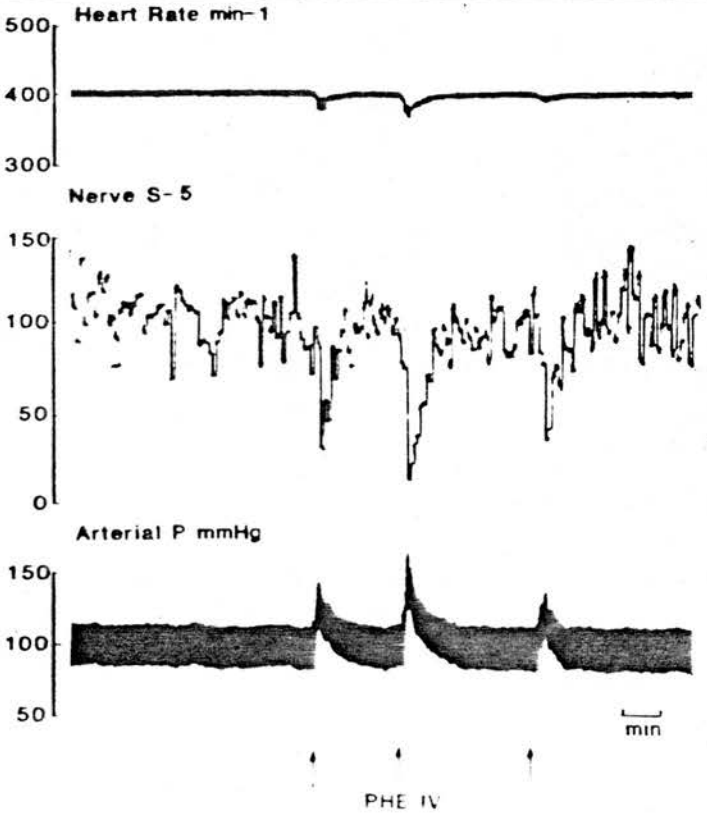


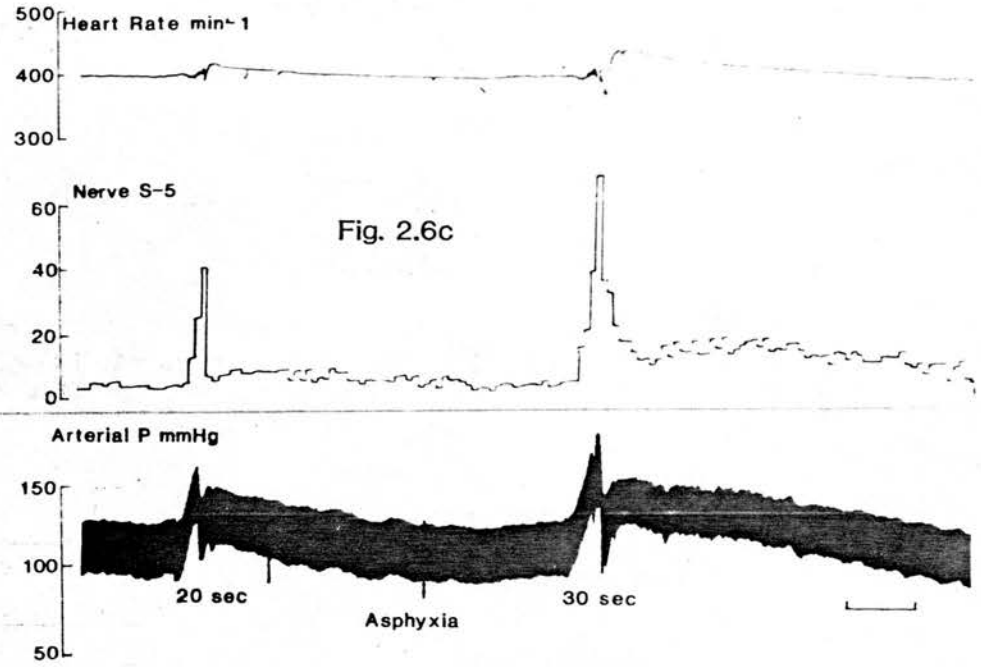
Fig. 2.6b

b) Phenylephrine bolus injection. The reduction in sympathetic avtivity is related to the magnitude of the increase in blood pressure as is the fall in heart rate. Recording from superior cervical nerve.

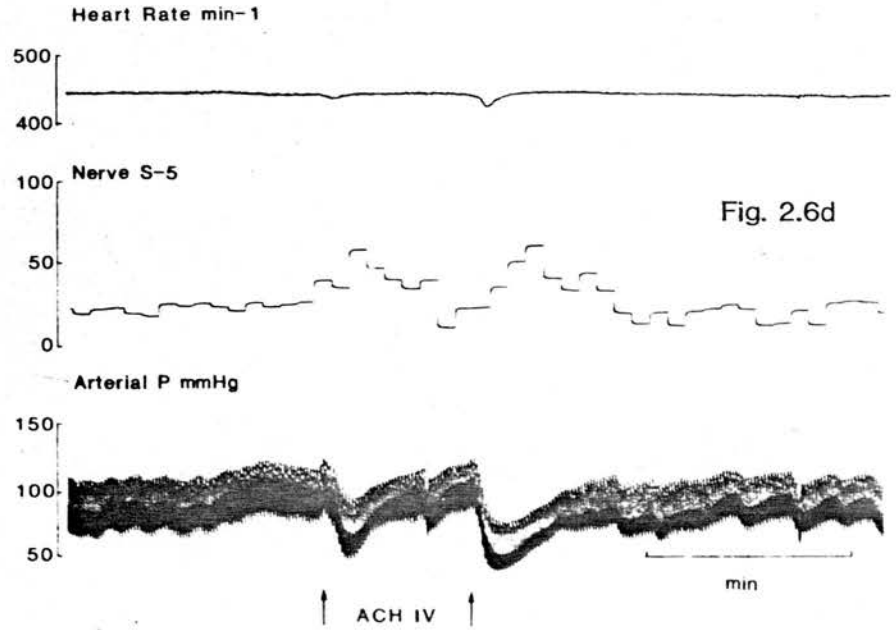
Fig. 2.6a,b,c,d

Sympathetic Efferent Activity In The Inactin Anaesthetized Rat

The counting method used, accumulation in one period and display on the trace in the next period, means that nerve activity refers to the previous time interval.



c) Asphyxia. The tracheal cannula was occluded to produce asphyxia. Blood pressure, heart rate and nerve activity all increase.

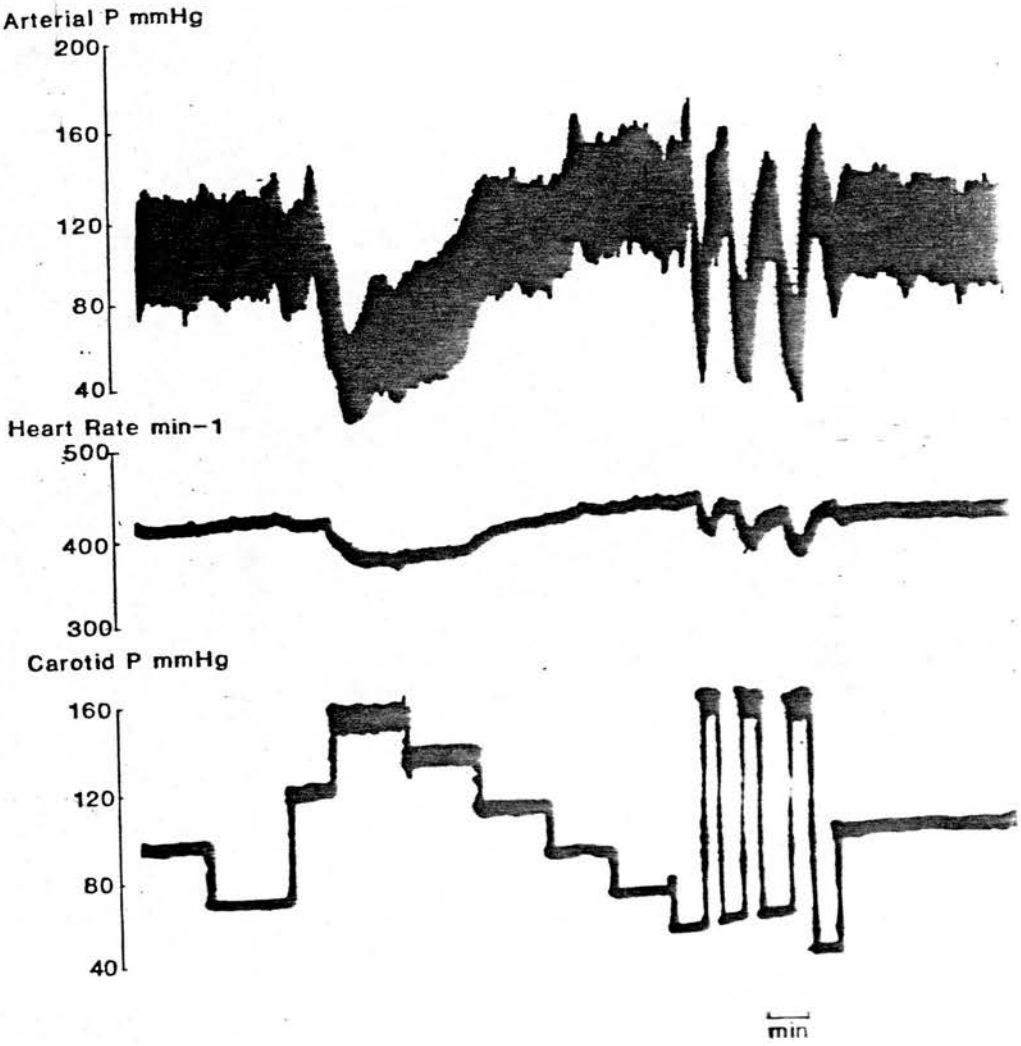


d) Acetylcholine IV. Blood pressure and heart rate fall whilst nerve activity increases. Recording from renal nerve.

Fig. 2.7

Changes in blood pressure and heart rate produced by the alteration in pressure around the carotid baroreceptors. This was achieved by cannulating both ends of a cut carotid artery andusing a roller pump to drive blood from the cardiac end of the artery to the cerebral end. The pump was controlled by a feedback loop allowing the generation of a fixed pressure downstream of the pump (see Appendix 1).

Note reduction in heart rate and arterial blood pressure when the carotid pressure is increased.



perfusion pressure. Fig. A6.b only shows alterations in femoral arterial pressure.

A similar experiment shown in Fig. A6.c used the feedback controller to infuse phenyephrine which increased arterial pressure and led to a corresponding fall in heart rate.

The results illustrated are anecdotal and this preparation was not pursued.

Discussion

Blood pressure recorded from the cranial end of a carotid artery follows arterial pressure recorded from the cardiac side of the same blood vessel, but at a much reduced level. It follows that one set of carotid sinus baroreceptors is exposed to a low pressure and the animal is probably functionally equivalent to continuous unilateral carotid occlusion. To avoid this the femoral artery was used for the arterial pressure cannula in all subsequent experiments. The Circle of Willis is unable to deliver sufficient blood to the side of the head deprived of its arterial supply and that side of the head is probably hypoxic (see autoradiographic evidence, chapter 5).

Clonidine reduced heart rate and blood pressure in a dose dependant manner over the range of doses used, 1-10 ug/Kg. This range was employed in later experiments, excluding higher concentrations that produce prolonged hypertensive episodes. In fig 2.4d the last two injections of clonidine resulted in a prolonged increase in arterial pressure. Two components are apparent one a rapidly rising and falling following immediately after IV adminstration of clonidine and a second hypertensive episode with a longer duration that appears as the initial peak declines. This pattern was seen in two of the animals pretreated with hexamethonium. The second hypertensive period may involve release of catecholamines from the adrenal medulla, reported by Zandberg (1977). That it occurred in a hexamethonium-pretreated animal requires that it involve a direct action of clonidine. Zandberg found that clonidine-evoked catecholamine release was reduced in barbiturate anaesthetized rats compared with urethane anaesthetized or conscious animals. The suggestion that it is occurring in the inactin anasthetized rat is speculative but the

prolonged duration of the pressor effect seems to be at odds with the very dramatic fall in plasma clonidine noted in chapter 6.

In the group of animals given 10 ug/Kg IV heart rate declines more slowly than mean arterial pressure, possibly indicating that the two effects are separate in some respects. It does not involve the brief pressor effect following bolus IV clonidine which in these experiments lasted less than one minute.

The cardiovascular actions of clonidine are maintained and only a partial recovery appeared in a group of animals followed for 55 mins after injection. This lack of acute recovery justifies the use of cumulative applications of clonidine in subsequent experiments.

Resting heart rate in the inactin anaesthetized rat appears to be under sympathetic but not vagal control, since atropine leaves the resting heart rate unaltered whilst atenolol reduced it by 60 beats per minute. This has been noted before in the pentobarbitone anaesthetized rat Imms et (1976). This may be in part an anaesthetic artefect since in the dog bradycardia due to sinus nerve stimulation switches from the vagal to sympathetic evoked after barbituate anaesthesia (Vatner et al 1971). The small variations in heart rate around the mean also appeared to be under sympathetic control since atenolol reduced them in all cases where they appeared. Atenolol led to only a small reduction in mean arterial pressure, around 2 mmHg and this suggests that heart rate is not an important controller of blood pressure in this preparation. The temporal dissociation of heart rate and blood pressure change seen with 10 mg/Kg clonidine IV supports this idea.

When all nervous influences on heart rate are removed, by hexamethonium, the residual rate is mainly that arising from the cardiac pacemaker tissue. In these animals under inactin anaesthesia this varied from 310 to 415 min^{-1}.

Resting heart rate responds to vasopressor and vasodilator stimuli. The responses are in line with baroreflex actions, a decrease after phenylephrine and an increase following glyceryl trinitrate or acetylcholine. The reduction in mean arterial pressure following clonidine would be expected to lead to a reflex increase in heart rate. That this does not occur shows that clonidine alters the operation of the baroreflex arc.

Clonidine has a much reduced action on heart rate after

atenolol pretreatment. After atropine the magnitude of the reduction is similar to that seen without atenolol suggesting that its cause is a reduction in sympathetic tone. Although failing to alter heart rate after atenolol pretreatment clonidine still lowered blood pressure again showing the separation between these two effects. When the cumulative dose of clonidine was of sufficient magnitude that no further fall in heart rate or arterial pressure appeared, a fall in heart rate occured during the brief hypertensive period after each bolus IV injection. It also appeared after pretreatment with atenolol which shows that a non-sympathetic pathway is implicated, either a direct action or mediated through the vagus. Ths could be seen as the enhancement of the baroreflex commonly seen in dogs after the administration of clonidine.

The failure of atropine to alter heart rate after the administration of clonidine and atenolol indicates that there is no vagal cardiac tone. Similarly atenolol does not further reduce heart rate when administered after clonidine and atropine, showing that sympathetic tone does not contribute to the final magnitude of heart rate. When sufficient clonidine has been administered the heart is devoid of all nervous tone, but acute increases in arterial pressure can still evoke a further fall in rate. This must be attributed to a vagal influence since the phenomena appears after atenolol pretreatment which abolishes sympathetic tone.

Clonidine reduces peripheral resistance in the perfused hindquarters and hindlimb. As the cumulative clonidine levels increase peripheral resistance and arterial pressure cease falling and increase. Heart rate does not increase. The technique employed does not indicate whether nervous or humoral factors are involved, this is further pursued in the next chapter. Since a pressor action with clonidine appears where neural autonomic control is removed using hexamethonium, this action is peripheral in origin. In the pithed rat pressor responses to clonidine are obtained. They occur with concentrations above those needed to alter transmitter release from sympathetic nerve terminals, in clinical use the application of large quantities of clonidine can lead to a paradoxical increase in mean arterial pressure (Wing et al 1977, Saarima 1976).

In the inactin anaesthetized rat cardiovascular manipulations led to alterations in sympathetic efferent activity. This suggests

that the use of this anaesthetic in the investigation of cardiovascular reflexes is feasible. It had been intended initially to study the effect of clonidine on functionally identified sympathetic efferent nerves but the difficulties in obtaining stable single unit recordings led to the adoption of the "delayed" hindlimb perfusion technique. This allowed the visualization of neural control of the peripheral vasculature.

Tables for Chapter 2

The Action of Atropine and Atenolol on Blood Pressure and Heart Rate

Atropine 1 mg/Kg IV

Time mins	Heart Rate (min-1)							
10	-3	0	1	5	-4	-3	0	-2
-5	-5	-7	0	9	-9	-1	0	-2
-2	-6	-6	2	4	-2	1	1	-2
-1	-6	-4	0	3	0	0	0	-2
0	421	349	407	372	383	503	444	412
1	1	0	-2	-1	4	3	1	-6
2	-2	1	-2	-2	7	0	-2	-8
5	-8	2	-2	-4	-1	-6	-4	-8
10	-17	3	-7	-2	5	-6	-5	-14

	Blood Pressure (mmHg)							
-10	0.5	-2	-1	2.5	3.5	4.5	0	1
-5	0.5	-6	0	-1	-3	4.5	-1	1
-2	0.5	-4	0	-0.5	-0.5	2.5	1.5	1
-1	-2.5	-2	0	0	0	0.5	0.5	0
0	91.5	113	91	88.5	90	106	94	103
1	-0.5	-1	-4	-2	-4	-5	0	-5.5
2	-0.5	0	-4	-2	-1.5	-5	0	-6
5	1.5	1	-4	0.5	0	-10	1.5	-6.5
10	4.4	-2	-3	3	0	-7.5	2	-7.5

Atenolol 1 mg/Kg IV

Time mins	Heart Rate (min-1)					
-10	0	-2	-4	-2	-1	4
-5	0	-2	-6	-2	2	2
-2	0	-1	0	-4	1	0
-1	0	0	0	-2	0	0
0	376	416	436	396	354	435
0.5	-36	-26	-60	-26	-24	-10
1	-54	-30	-68	-40	-34	-15
2	-58	-40	-78	-44	-37	-29
5	-64	-56	-88	-48	-36	-47
10	-68	-56	-90	-48	-44	-47

	Blood Pressure (mmHg)					
-10	0	2.5	-1	2	1.5	0
-5	2	2.5	1	2	1	0
-2	1.5	-0.5	0	1.5	1	0.5
-1	0.5	0	0	1.5	1	1
0	114.5	119	94	75	102.5	116
1	-2	-1	-3.5	0.5	-3.5	6.5
2	-3	-0.5	-4.5	0	-1.5	3
5	-4	-4	-5.5	0	0	2
10	-5	-14	-4.5	1.5	-9	1

The Action of Clonidine on Heart Rate After pretreatment with Atropine or Atenolol

Cumulative Clonidine IV After Atropine

Clonidine ug/Kg	Heart Rate (min-1)							
0	400	402	387	398	439	363	483	356
2.5	-48	-25	-25	-43	-83	-30	-68	-24
5	-50	-27	-23	-54	-103	-30	-88	-25
7.5	-48	-29	-23	-61	-117	-30	-95	-30
10	-42	-33	-22	-66	-123	-30	-119	-30
12.5	-38	-36	-24	-72	-131	-30	-129	-35
15	-32	-40	-27	-72	-133	-30	-143	-38

	Blood Pressure (mmHg)							
0	119	106	90	90	96	91.5	103	113
2.5	-8	-5.5	-0.5	-3	-7	-6	-6.5	-14
5	-11	-10.5	0	-7.5	-9	-9	-12	-25.5
7.5	-11.5	-12	0.5	-9	-12.5	-11.5	-15.5	-25
10	-11.5	-13	-0.5	-13.5	-13.5	-14.5	-16	-25
12.5	-13.5	-14	-2.5	-17.5	-13	-18.5	-16	-26
15	-11.5	-15	-5.5	-19	-12.5	-16.5	-16.5	-26

Cumulative Clonidine After Atenolol

	Heart Rate (min-1)					
0	318	360	346	350	300	435
2.5	2	-18	-4	-8	-9	-11
5	-6	-32	-6	-10	-10	-20
7.5	-7	-30	-8	-15	-10	-22
10	-8	-32	-6	-20	-9	-25
12.5	-7	-31	-1	-24	-9	-26
15	-6	-30	-3	-28	-9	-24

	Blood Pressure (mmHg)					
0	90	105	86	79	86.5	122.5
2.5	-14.5	-22.5	-7	-2	-8	-6.5
5	-21	-31.5	-11	-3	-11.5	-10
7.5	-25	-34.5	-16	-4	-16	-11
10	-28	-34.5	-19.5	-5	-19	-12.5
12.5	-30	-34.5	-20.5	-5	-17	-13
15	-34.5	-34.5	-20.5	-5.5	-15	-13

The Effect of Intravenous Clonidine on Blood Pressure and Heart Rate

Clonidine IV 1 ug/Kg

Time min	Heart Rate min-1					
-10	-8	-2	-10	-10	3	17
-5	-10	-2	-18	-13	0	5
-4	-6	1	-17	-8	2	3
-3	-4	1	-12	-4	3	1
-2	-2	0	-6	-2	4	1
-1	0	-2	-2	-2	0	0
0	404	358	402	364	431	425
1	-16	-14	-17	-14	-21	-17
2	-20	-15	-12	-13	-28	-20
3	-21	-14	-9	-9	-32	-25
4	-23	-14	-8	-4	-33	-25
6	-30	-8	-3	-4	-38	-27
8	-36	-4	40	-15	-39	-29
10	-39	-2	4	-24	-42	-31
15	-41	7	-6	-24	-46	-36
20	-43	2	-17	-17	-42	-36
25	-51	-7	-32	-11	-35	-35
30	-53	-18	-37	-10	-38	-29

	Blood Pressure mmHg					
-10	0	2	-2	0	-1	5
-5	0	2	-3	0	-2	3
-4	0	1	-4	0	-1	2
-3	0	1	-3	0	-1	4
-2	0	0	-3	0	0	0
-1	0	0	0	1	-1	1
0	95	102	79	79	108	91
1	-2	-3	-4	-2	-5	-2
2	-5	-4.5	-3	-3	-7	-3
3	-4	-5	-3	-3	-7	-4
4	-3.5	-5	-1	-3	-7	-5
6	-6.5	-5.5	-1	-2	-7	-4
8	-7.5	-5	-2	-3	-7	-4
10	-8.5	-5.5	-3	-4	-7	-7
15	-13	-7.5	-4	-4	-7	-8
20	-16.5	-11.5	-8	-5	-7	-8
25	-19	-15	-9	-3	-7	-8
30	-20.5	-19	-9	-4	-6	-6

Clonidine IV 2.5 ug/Kg

Time	Blood Pressure										
mins	mmHg										
-10	-4	1	1	3	6	-3	5	-1	2	-1	3
-5	-5	1	0	-2	2	-1	5	-1	2	2	1
-4	-5	2	-1	-3	2	-2	2	-1	3	0	1
-3	-5	0	-1	-2	2	-1	1	0	3	0	1
-2	-5	0	1	-3	1	-1	1	0	4	1	1
-1	-1	-1	1	1	0	-1	0	0	2	0	1
0	99	82	92	87	103	104	101	102	87	88	101
1	-7	-8	-2	-9	-10	-5	-6	-6	-9	-16	-11
2	-11	-7	-5	-11	-11	-6	-8	-10	-14	-23	-13
3	-11	-7	-6	-9	-12	-6	-9	-10	-17	-29	-18
4	-11	-7	-8	-11	-12	-6	-9	-11	-15	-28	-22
6	-12	-6	-7	-11	-16	-7	-10	-11	-18	-28	-21
8	-10	-6	-8	-11	-14	-6	-13	-12	-21	-28	-23
10	-9	-8	-9	-13	-12	-4	-14	-12	-20	-26	-24
15	-2	-8	-5	-12	-12	-1	-13	-12	-19	-25	-25
20	-6	-8	-3	-9	-13	3	-13	-12	-22	-17	-27
25	-7	-7	-3	-8	-13	4	-11	-12	-17	-14	-27
30	-8	-8	0	-6	-12	4	-3	-11	-15	-13	-26
35	-7	-8	-1	-5	-12	3	2	-12	-12	-10	-26

	Heart Rate										
	min-1										
-10	12	-13	-8	-1	13	-17	10	2	0	-5	0
-5	5	-5	-4	-1	5	-6	13	1	0	-7	0
-4	5	-3	-1	0	4	-7	9	0	0	0	0
-3	1	-3	-2	0	2	-4	8	0	0	-2	0
-2	1	-1	2	-1	1	-2	3	0	0	-2	0
-1	0	-3	2	0	0	0	1	0	0	0	0
0	485	453	435	407	467	427	447	400	375	370	380
1	-23	-18	-37	-6	-39	-11	-39	-45	-45	-50	-50
2	-27	-15	-38	-11	-50	-12	-45	-55	-60	-60	-66
3	-27	-11	-38	-12	-53	-16	-47	-60	-67	-65	-65
4	-25	-9	-35	-17	-52	-21	-51	-65	-67	-72	-70
6	-17	-5	-39	-17	-54	-24	-59	-62	-80	-77	-78
8	-13	-3	-43	-17	-56	-27	-61	-62	-86	-77	-82
10	-1	1	-47	-18	-57	-27	-61	-60	-85	-77	-87
15	19	0	-27	-18	-46	-30	-61	-58	-83	-75	-92
20	17	0	0	-17	-42	-29	-61	-58	-83	-75	-92
25	10	0	-4	-17	-36	-31	-61	-58	-80	-70	-90
30	1	0	6	-17	-30	-31	-35	-55	-75	-65	-87
35	-3	0	8	-15	-25	-30	-16	-50	-67	-60	-83

Clonidine IV 5 ug/Kg (1)

Time	Blood Pressure						
min	.....			mmHg	.....		
-10	2	3	2	3	4	4	0
-5	1	2	2	3	4	2	0
-4	2	1	1	1	3	1	1
-3	1	0	1	1	-1	2	1
-2	0	2	1	0	0	1	0
-1	0	1	1	0	0	0	1
0	93	88	105	132	108	89	96
1	-11	-9	-10	-17	-30	-10	-9
2	-15	-16	-15	-20	-27	-19	-10
3	-14	-14	-16	-20	-25	-20	-12
4	-15	-18	-17	-21	-23	-20	-14
6	-16	-16	-18	-23	-24	-23	-17
8	-15	-17	-18	-26	-27	-24	-19
10	-15	-16	-21	-29	-28	-24	-21
15	-18	-15	-17	-31	-27	-25	-25
20	-14	-14	-17	-28	-33	-25	-25
25	-16	-16	-14	-27	-36	-24	-25
30	-16	-16	-12	-27	-40	-20	-26
35	-16	-16	-11	-27	-39	-16	-27

	Heart Rate						
	.....			min-1	.....		
-10	-10	21	16	–	–	–	–
-5	-2	10	9	–	–	–	–
-4	0	7	4	–	–	–	–
-3	0	0	3	–	–	–	–
-2	0	7	3	–	–	–	–
-1	0	0	1	–	–	–	–
0	400	410	407	–	–	–	–
1	-38	-36	-37	–	–	–	–
2	-38	-46	-45	–	–	–	–
3	-50	-47	-48	–	–	–	–
4	-44	-46	-49	–	–	–	–
6	-45	-43	-56	–	–	–	–
8	-44	-40	-58	–	–	–	–
10	-37	-35	-60	–	–	–	–
15	-25	-30	-56	–	–	–	–
20	-40	-37	-42	–	–	–	–
25	-50	-44	-26	–	–	–	–
30	-50	-47	-22	–	–	–	–
35	-50	-38	-17	–	–	–	–

Clonidine IV 5 ug/Kg (2)

Time min	Blood Pressure mmHg						
-10	-1	4	0	1	1	-1	0
-5	1	2	0	1	1	1	1
-4	-1	1	0	1	0	1	-1
-3	0	0	0	1	0	0	0
-2	0	0	0	2	-0. 5	0	0
-1	0	0	0	0	0	0	0
0	103	104	102.5	100	81	100	102
1	-11	-7	-9.5	-11	-8.5	-13	-44
2	-18	-7	-13.5	-13	-13	-15	-48
3	-18	-7	-14	-17	-14	-17	-48
4	-19	-7	-15	-20	-15	-21	-47
6	-20	-7	-16	-26	-16	-23	-45
8	-20	-10	-16	-28	-17	-23	-41
10	-21	-12	-16.5	-31	-18	-23	-46
15	-23	-12	-17	-29	-18	-23	-36
20	-27	-12	-17.5	-27.5	-18	-21	-36
25	-26	-10	-16	-28	-18	-20	-34
30	-25.5	-8	-15.5	-28	-18	-19	-34
35	-26	-6	-16	-27	-16	-18	-32

	Heart Rate min-1						
-10	—	—	—	—	—	0	0
-5	—	—	—	—	—	0	0
-4	—	—	—	—	—	0	0
-3	—	—	—	—	—	0	0
-2	—	—	—	—	—	0	0
-1	—	—	—	—	—	0	0
0	—	—	—	—	—	348	400
1	—	—	—	—	—	-38	-50
2	—	—	—	—	—	-53	-70
3	—	—	—	—	—	-58	-80
4	—	—	—	—	—	-60	-85
6	—	—	—	—	—	-68	-82
8	—	—	—	—	—	-70	-85
10	—	—	—	—	—	-76	-87
15	—	—	—	—	—	-78	-90
20	—	—	—	—	—	-78	-92
25	—	—	—	—	—	-78	-95
30	—	—	—	—	—	-75	-95
35	—	—	—	—	—	-73	-85

Clonidine IV 10 ug/Kg (1)

Time						
min	Blood Pressure mmHg					
-10	-1	4	10	5	0	2
-5	1	3	5	3	0	2
-4	1	1	5	1	0	2
-3	0	-1	3	2	1	1
-2	-1	0	2	1	0	1
-1	1	-1	1	0	0	1
0	107	89	103	110	94	87
1	-16	-11	-18	-44	-12	-15
2	-21	-15	-31	-48	-17	-20
3	-23	-17	-36	-42	-20	-20
4	-25	-18	-37	-36	-22	-19
6	-27	-17	-36	-36	-24	-20
8	-29	-18	-36	-40	-25	-21
10	-28	-18	-37	-38	-25	-21
15	-26	-17	-38	-37	-25	-23
20	-26	-16	-38	-40	-22	-23
25	-24	-11	-36	-37	-16	-20
30	-24	-11	-35	-38	-15	-17
35	-24	-10	-34	-39	-14	-18
40	-26	-11	-29	-39	-16	-17
45	-25	-10	-25	-38	-16	-16
	Heart Rate min-1					
-10	-6	23	-7	-1	-3	10
-5	0	12	-9	-4	-1	6
-4	-1	3	-7	-6	1	5
-3	0	1	-5	-2	2	2
-2	0	0	-3	0	2	4
-1	0	1	0	0	1	1
0	427	442	415	404	413	441
1	-58	-45	-42	-42	-46	-52
2	-69	-54	-57	-59	-55	-58
3	-70	-58	-60	-63	-58	-64
4	-74	-61	-64	-67	-62	-71
6	-82	-64	-73	-72	-68	-76
8	-93	-67	-74	-77	-70	-80
10	-92	-66	-77	-81	-73	-85
15	-84	-63	-75	-88	-75	-100
20	-81	-63	_	-72	-69	-110
25	-80	-62	_	-54	-53	-111
30	-80	-59	_	-37	-46	-102
35	-78	-57	_	-39	-45	-83
40	-79	-56	_	-44	-50	-69
45	-78	-56	_	-44	-58	-53

Clonidine IV 10 ug/Kg (2)

time	Blood Pressure					
min	.....mmHg.....					
-10	-7	-1	-1	2	1	0
-5	-7	-1	-2	-1	0	0
-4	-8	1	-2	0	-2	0
-3	-7	-1	-1	1	-2	0
-2	-5	-1	0	1	-2	0
-1	3	0	-1	0	0	0
0	140	101	122	121	98	115
1	-15	-42	4	1	-42	-52
2	-19	-45	-4	-12	-53	-50
3	-23	-54	-11	-17	-53	-47
4	-25	-57	-14	-20	-48	-47
6	-26	-56	-18	-25	-33	-48
8	-26	-58	-21	-31	-32	-48
10	-22	-59	-23	-37	-34	-31
15	-21	-60	-25	-37	-39	-39
20	-21	-61	-23	-35	-42	-40
25	-21	-60	-23	-35	-44	-43
30	-25	-60	-21	-37	-47	-40
35	-25	-58	-19	-39	-51	-34
40	-25	-57	-18	-40	-53	-45
45	-24	-57	-16	-40	-51	-45

	Heart Rate				
	.....min-1.....				
-10	_	-5	0	-5	-3	0
-5	_	5	0	-5	-3	0
-4	_	3	0	-5	-3	0
-3	_	0	0	0	0	0
-2	_	0	0	0	0	0
-1	_	0	0	0	0	0
0	_	365	332	390	403	475
1	_	-65	-50	-58	-63	-105
2	_	-80	-60	-62	-63	-110
3	_	-87	-62	-60	-63	-120
4	_	-95	-55	-60	-63	-120
6	_	-100	-55	-65	-78	-125
8	_	-102	-55	-65	-83	-135
10	_	-108	-62	-70	-88	-125
15	_	-122	-64	-65	-93	-140
20	_	-133	-67	-57	-98	-155
25	_	-140	-72	-58	-103	-160
30	_	-143	-72	-58	-103	-165
35	_	-143	-70	-60	-103	-160
40	_	-143	-70	-68	-103	-155
45	_	-143	-70	-68	-103	-120

"Delayed" Hindlimb Perfusion

In the previous chapter clonidine was shown to reduce vascular resistance in the hindlimb and hindquarters. Changes in vascular resistance shown in constant rate or pressure perfusions may involve:

1) direct action on the vasculature by blood borne agents.
2) changes in vasoconstrictor or vasodilator nerve tone.
3) action by blood borne agents on neurotransmitter release, uptake or metabolism.
4) autoregulatory changes.
5) a net response of different portions of the vasculature.

The interpretation of the mechanisms involved is therefore difficult. To improve the utility of perfusion experiments the vasculature may be denervated, cross circulation techniques employed or drugs may be administered by close arterial injection.

Cross circulation experiments involve pumping blood from a donor into the arterial supply of a vascularly isolated tissue and returning the venous effluent to the donor. Changes in resistance and capacitance represent nervous or blood-borne responses depending on whether the stimulus is applied to the donor or the recipient. An important variation utilises a vascularly isolated head and allows separation of central from peripheral responses (Nowak et al 1935 for historical review). Taylor et al (1951) and Bickerton et al (1961) perfected the technique with a relatively simple method for occluding the venous sinuses under the ventral spinal cord but the authors reported difficulty in maintaining the experimental animals over several hours. Cerebral cross circulation experiments have not been reported in rats probably because of the low historical cost of dogs and the intricacy of the surgery.

Field et al (1958), Clark et al (1975) and Sakai (1978) report using cross circulation procedures in the rat hindlimb. Returning venous blood to the donor whilst maintaining venous pressure constant appears to be a problem. Field and Sakai (1978) used a venous reservoir and intermittent pumping whilst Clark used a second pump without mention of a reservoir and may have encountered problems with two pumps operating in series. A progressive build up

Fig. 3.1a

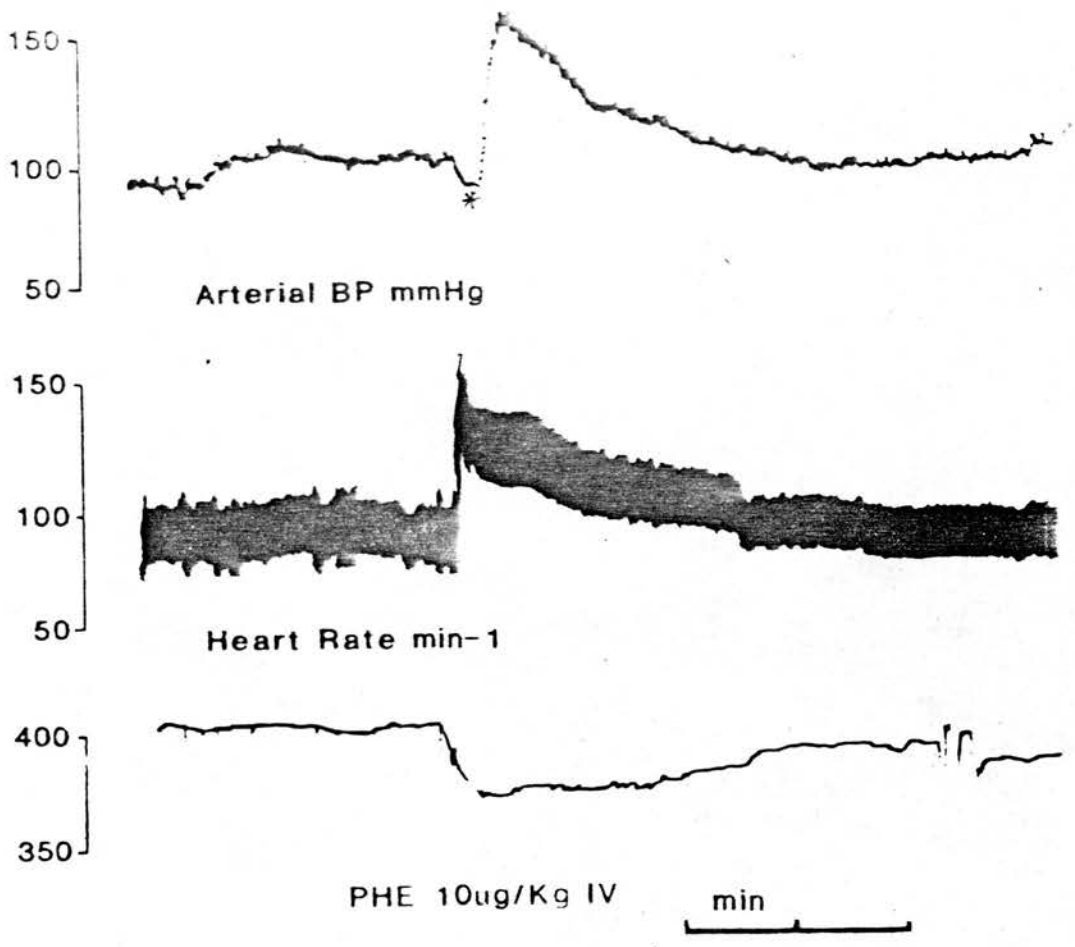


Fig. 3.3b

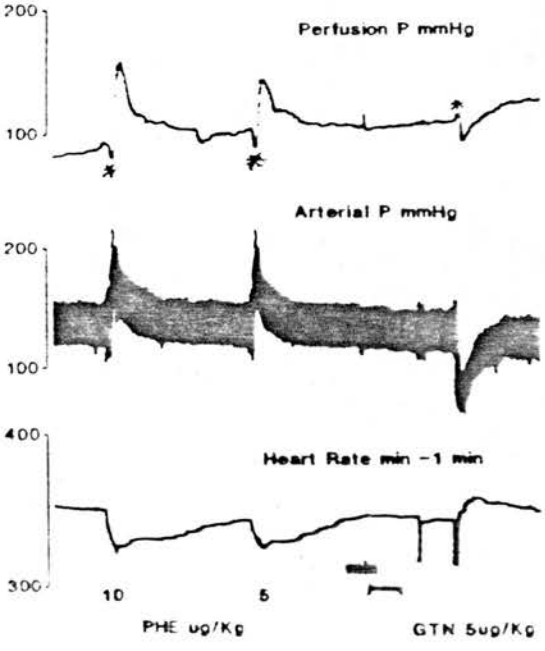


Fig. 3.3c

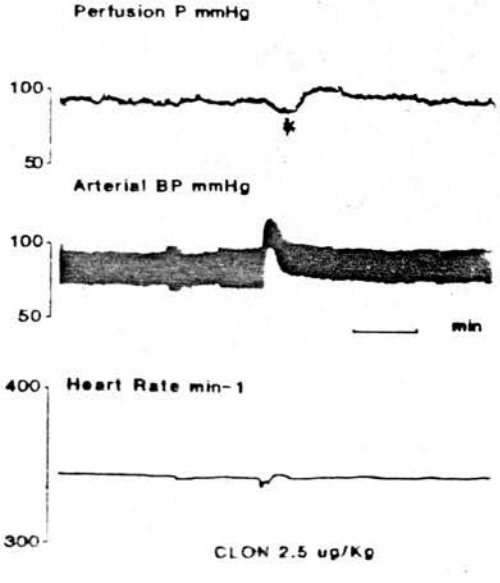


Fig. 3.1.
"Inappropriate" responses to clonidine, glyceryl trinitrate and phenylephrine in the perfused rat hindlimb.
* marks responses of interest.

a) Phenylephrine 10 ug/Kg IV. Top Left.
Causes an initial brief fall in perfusion pressure in the perfused hindlimb, an unexpected effect for a vasoconstrictor drug. The expected pressor effect follows of similar duration in both hindlimb and on arterial blood pressure, the fall in heart rate mirrors the increase in arterial pressure.

b) Phenylephrine 5 and 10 ug/Kg IV and Glyceryl trinitrate 10 ug/Kg IV. Bottom Far Left.
As in a) a brief dilator action appears immiediately after phenylephrine. This unlike the drop in heart rate and increase in arterial pressure is unexpected. The slight increase in peripheral resistance seen with glyceryl trintrate is similarly unexpected as the drug is a vasodilator.

c) Clonidine 2.5 ug/Kg IV. Bottom Left.
The initial dilator effect in the hindlimb during the rise in arterial pressure is unexpected from a drug with vasoconstrictor actions after bolus IV administration.

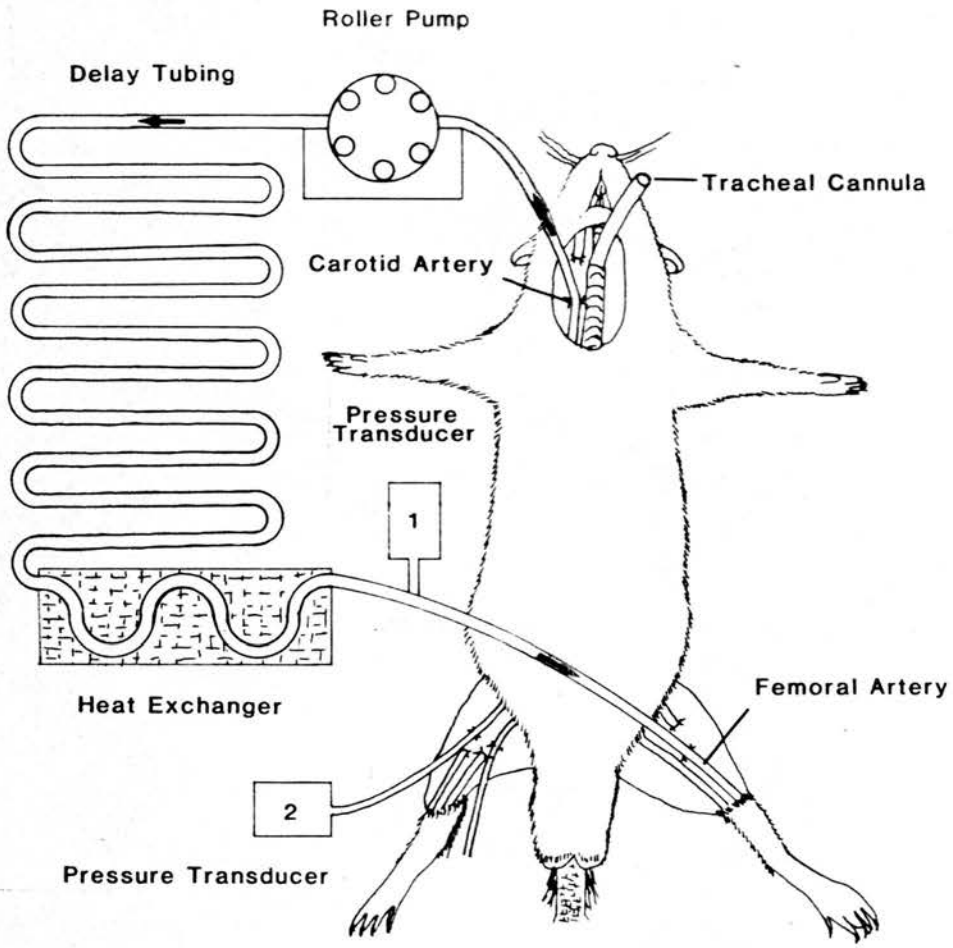
of fluid occurrs in part of the circuit if the rates of each pump differ however slightly. Clark (1975) however avoids using a third rat to fill the venous reservoir. A plausible solution requiring two rats could involve using the second roller pump with a feedback loop maintaining pressure downstream of the pump and by recording the pumping rate capacitance changes could be ascertained.

This solution was not possible with the equipment availible and a novel "delayed" hindlimb perfusion was developed. In some of the hindlimb perfusion experiments blood temperature was considered a problem and a heat exchanger was incorporated, necessitating an increase in the extracorporeal volume. This slighly delayed the arrival of blood-borne agents at the perfused hindlimb with respect to the remainder of the vasculature. Intravenously applied drugs produced "inappropriate" responses over this brief period, glyceryl trinitrate producing vasoconstriction, phenylephrine and clonidine vasodilation, before the expected effects manifested themselves, fig 3.1 a,b,c. The initial changes in perfusion pressure were interpreted as being nervous in origin, rather than direct actions of the drug, as the extracorporeal circuit has to be negotiated before blood-borne agents can act on the perfused tissue. Vasodilatation following clonidine or phenylephrine are then reflex responses to the pressor actions apparent in the arterial blood pressure trace. A method therefore presented itself for visualizing nervous influences on the vasculature. The preparation was developed by increasing the extracorporeal volume to between three and five mls. giving a delay of at least one and a half minutes, fig 3.2.

Preparation

It is designed to distinguish between acute changes in peripheral resistance mediated by humoral agents and those under nervous control. Blood-borne responses are delayed because the large external circuit takes time to negotiate and blood originating from the pre-stimulus state is in the meantime perfusing the hindlimb. There is no delay for nervously mediated effects and until post stimulus blood reaches the hindlimb nervous effects are seen in isolation. Changes seen concomitantly in arterial blood pressure represent an amalgam of the applied stimulus and reflex responses. After a delay vasoactive agents reach the hindlimb and their effect

Fig. 3.2
"Delayed" Hindlimb Perfusion



The hindlimb is perfused with blood drawn from the carotid artery. It passes through a long cannula containing several millilitres of blood and its arrival at the hindlimb is delayed, allowing neural responses to appear in the hindlimb separate from blood born effects. A heat exchanger positioned just before the blood returns to the animal restores the temperature of the perfusate to body temperature.

is superimposed on the existing level of nervous vasomotor tone. If the cardiovascular effects of the initial stimulus have abated and a new stable level of vasomotor tone become established then the delayed response only reflects direct actions on the vasculature. Finally changes in perfusion pressure represent a balance between nervous and blood-borne responses.

It is a common practice to record the activity in sympathetic efferent nerves and relate this to vasomotor tone. This requires that sympathetic efferents are homogenous, if not in actions at least in response. This is not justified as the work of Grosse & Janig (1976) illustrates. Sympathetic efferents show different response patterns therefore to use gross nerve activity as a measure of vasomotor tone is unjustified. The "delayed hindlimb" avoids this problem and alterations in vasomotor tone are seen without contamination from sudomotor and piloerector activity. The preparation does not differentiate between decreases in vasoconstrictor activity and increases in vasodilator tone. Further the vasculature studied involves muscles, skin and the foot with the net response appearing. The foot could have been ligated to exclude its involvement but was not, likewise the skin could have been removed.

Blood volume in rats has been estimated at between 4-11 ml/100 gm (Muelheims et al 1959, Wang 1959) therefore an extracorporeal circuit of 3-5 mls represents a substantial proportion of the blood volume. If uncompensated for the volume required to fill the external circuit would represent a substantial haemorrhage. To avoid using a second rat a range of plasma substitutes were tried: saline, Dextran 70, Haemacel. Replacement of both oxygen carrying capacity and blood volume are required. Saline having no osmotic activity proved to be of only short term utility. Dextran 70 was suprisingly ineffective, a subsequent literature review revealed an analphylactic reaction in rats (Edlund et al 1952, Ankier & Starr 1967, Voorhess et al 1951). A retrospective look at earlier experiments where dextran was used to increase blood pressure showed it to be of questionable utility. Haemacel (Hoechst), a gelatin based polymer with a molecular weight of around 37 000, reported to have fewer allergic effects, (Isbiter & Fisher 1980), did not appear of great utility, although its use was not systematically

investigated. Rather than invest a lot of effort in looking for blood substitutes it was decided to use blood from a second animal.

Delay times were calculated from the volume of the external circuit and tested using coloured solutions. The time taken for the dye to appear at the outflow after a switch from saline to coloured solution was shorter by several seconds than that calculated. This is probably accounted for by diffusion along the tubing. To minimise this PP50 polythene tubing was used, it has a narrow bore which reduced the time difference.

The utility of the technique requires that the vascular bed supplied by the femoral artery does not recieve a blood supply from another artery. If this were the case immediate changes is perfusion pressure could result from vasoactive agents delivered by a collateral supply and not as the techniques requires from nervous sources. To validate the technique perfusion pressures were measured after stopping the pump, and were around 10 mmHg. If a second arterial supply existed a higher pressure would be expected. Further cutting the femoral vein after ligating the femoral artery does not lead to sustained bleeding suggesting that the hindlimb blood supply is predominantly derived from the femoral artery in these preparations

Methods

1) Rats 250-300 gm prepared in the standard manner, tracheal cannulation, femoral artery and vein of one leg cannulated for IV injection and BP recording.

2) IV heparin 500 units. Carotid artery exposed, tied centrally and cannulated on the cardiac side.

3) The other femoral artery tied centrally and cannulated peripherally, taking care to avoid the adjacent nerve.

4) The perfusion apparatus filled with blood from the carotid artery of a donor rat, 150-200 gm anaesthetized IP inactin and heparinized.

5) The carotid artery and femoral arterial cannulae connected to appropriate ends of the perfusion apparatus and the perfusion started. After a few minutes the perfusion speed was adjusted to provide a perfusion pressure approximating to the arterial BP.

6) A heat exchanger containing water at 38°C kept the perfusate at body temperature. Warm water was obtained from a thermostatically controlled water bath and pumped through the heat exchanger.

Heparin was given regularly, 200 units every hour. It is important to fill the perfusion system with blood only when the experimental animal is fully prepared. Otherwise the RBCs in the tubing sediment and blockage of the perfusion system may occur. In several experiments the perfusion pressures increased progressively, suddenly returned to the initial level and then repeated the cycle. It was assumed that a blood clot had lodged in the vasculature leading to a build up of pressure which momentarily displaced the obstruction and allowed blood to pass. The appearance of this phenomenon signalled the useful end of an experiment.

Experiments

1) Responses to a variety of stimuli. Asphyxia, IV glyceryl trinitrate, carotid occlusion, IV noradrenaline, IV isoprenaline and IV phenylephrine were used to validate the technique and illustrate reflex responses in the inactin anaesthetized rat.

2) Responses to IV clonidine. Looking for changes in peripheral resistance resulting from reduced vasomotor tone and the direct actions of clonidine on the hindlimb. The combined response was studied after 9 mins when changes in peripheral resistance are due a combination of central and peripheral actions, to asess the importance of nervous and direct effects.

Results

1) Various Stimuli.

These are typical responses,they were not sytematically applied but used to establish that the preparation was functioning.

a) IV Phenylephrine, Fig. 3.3 a,b. An immediate increase in arterial blood pressure and fall in heart rate and vascular

Fig. 3.3 a,b

The "Delayed" Hindlimb Perfusion With A Variety Of Stimuli.

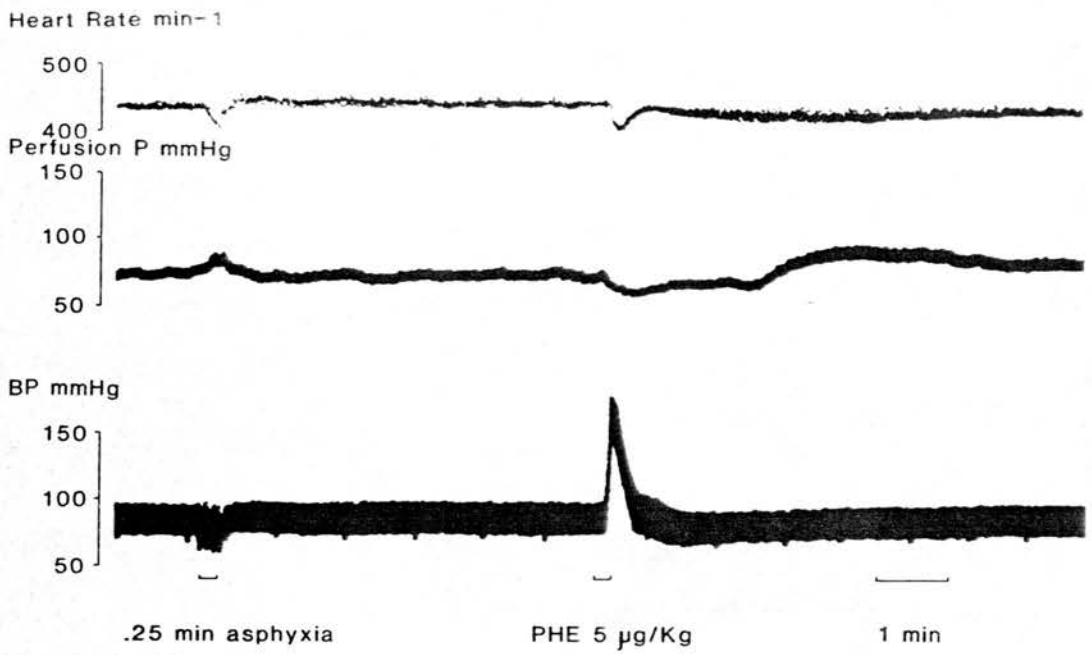


Fig. 3.3 a

1/4 Minute Asphyxia And Phenylephrine 5 ug/Kg IV

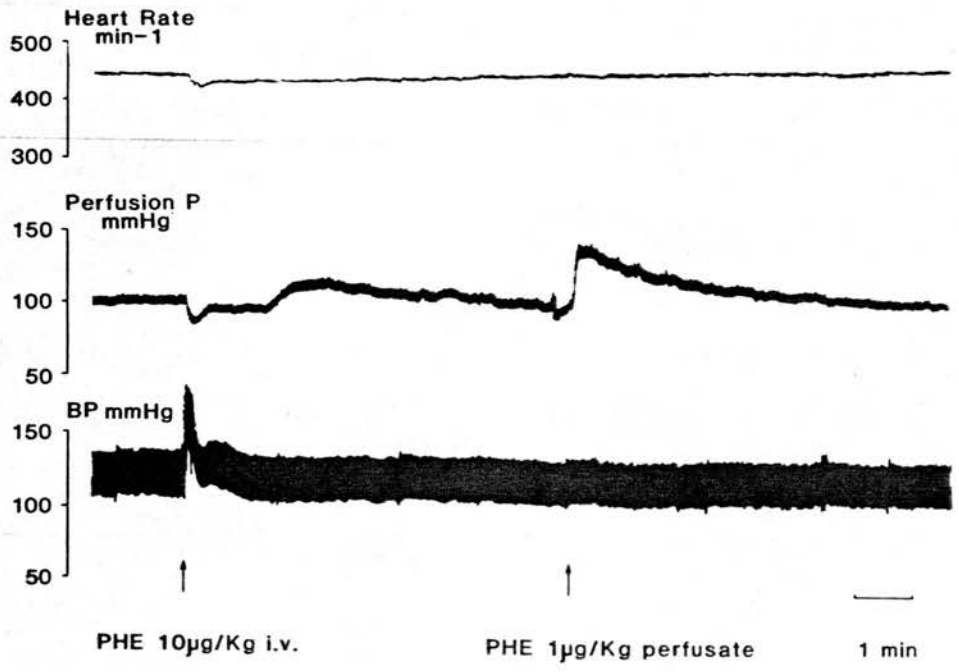


Fig. 3.3 b

Phenylephrine 10 ug/Kg IV And Phenylephrine Injected Into The Perfusate.

The latter initially delivers the drug only to the hindlimb.

resistance in the hindlimb. The delayed response was pressor and of greater duration than that seen in arterial pressure, though the rate of rise of pressure and peak effect were markedly reduced. Diffusion within the delay tubing would account for both phenomena, reducing the concentration peak following bolus IV administration and delivering phenylephrine over a longer period. An additional factor reducing the duration of the arterial pressor response is the neurally mediated reduction in peripheral resistanc, seen in the hindlimb and presumably occuring in other vascular beds.

b) IV Adrenaline Fig. 3.3 h. Similar responses to IV phenylephrine though no fall in heart rate occured.

c) IV Glyceryl Trinitrate Fig. 3.3 c,g. An immediate drop in arterial pressure, increase in heart rate and increase in hindlimb vascular resistance. Arterial pressure and heart rate return to preinjection levels and a delayed fall in resistance appears in the hindlimb. These results are compatible with a direct relaxant action for glyceryl trinitrate and reflex responses by the anaesthetized animal.

d) IV Isoprenaline Fig. 3.3 f. A vasodilator action explains the drop in arterial pressure and rise in hindlimb resistance that follow IV isoprenaline. The failure of heart rate to increase is surprising.

e). Asphyxia Fig. 3.3 a,e,g. Asphyxia was achieved by covering the tracheal cannula of spontaneously breathing animals. In each instance hindlimb resistance increases immediately, in Fig. 3.3 a arterial pressure did nor rise and in all three instances no increase in heart rate was seen. The rise is perfusion pressure is neural in origin and no delayed effect was seen. Possibly prolonging the asphyxia would produce a delayed dilatation.

f). Bilateral Carotid Occlusion Fig. 3.3 d. An immediate increase in arterial and hindlimb pressure resulted, heart rate did not rise but the fluctuations around the mean abated for the duration of the occlusion. Resting heart rate was high 493 min^{-1}.

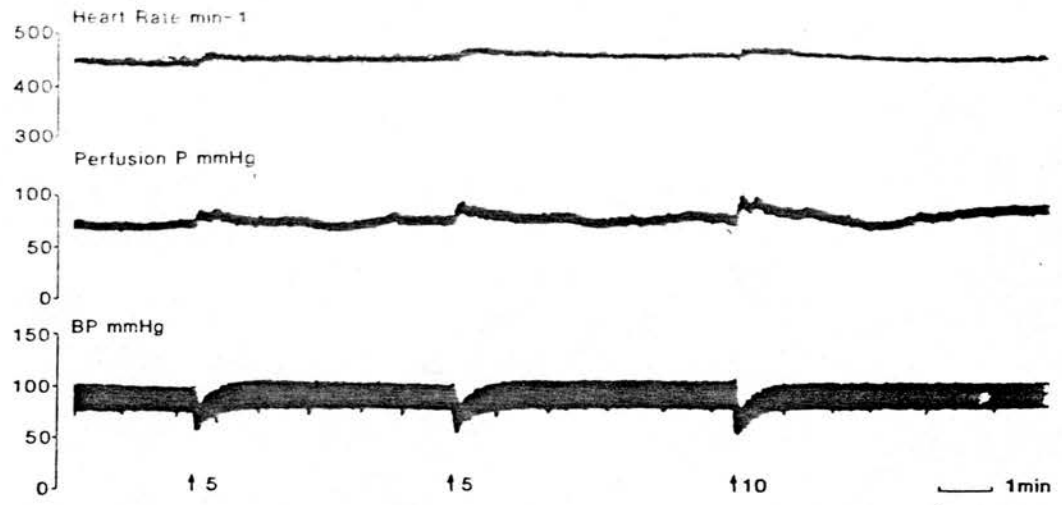


Fig. 3.3c

GTN IV µg/Kg

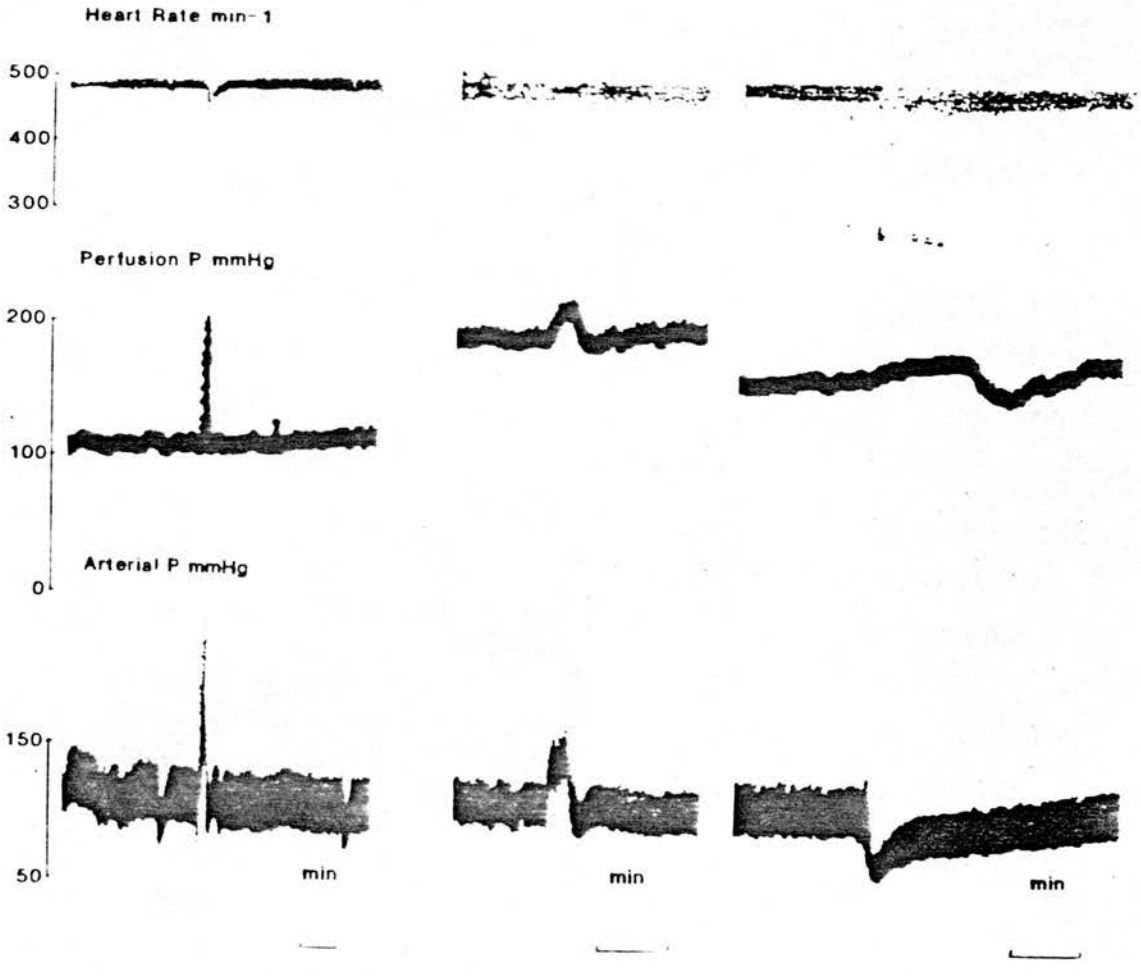


Fig. 3.3d

Fig. 3.3e

Fig. 3.3f

Fig. 3.3 c,d,e,f

The "Delayed Hindlimb Perfusion With A Variety Of Stimuli

Fig. 3.3 c

Glyceryltrinitrate IV

Perfusion pressure increases in the hindlimb whilst arterial blood pressure falls. A delayed decrease in perfusion pressure is apparent.

Fig. 3.3 d

Carotid Occlusion Far Left.

Arterial and perfusion pressure rise simultaneously.

Fig. 3.3 e

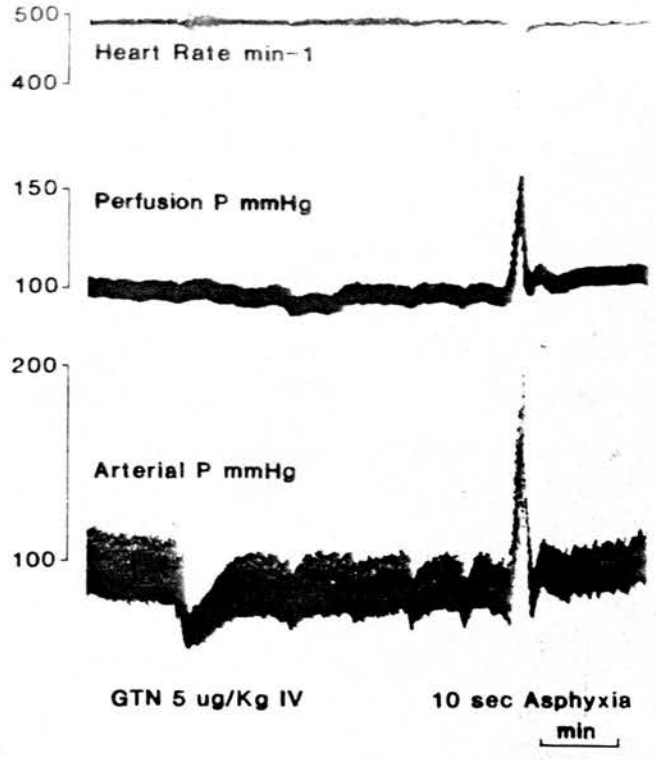
Asphyxia Middle Left.

Arterial and perfusion pressure rise in unison, there is no temporal separation.

Fig. 3.3 f Near Left

Isoprenaline IV

The fall in perfusion pressure occurs after that seen in arterial pressure.


500
Heart Rate min-1
400
150
Perfusion P mmHg
100
200
Arterial P mmHg
100
GTN 5 ug/Kg IV
10 sec Asphyxia
min

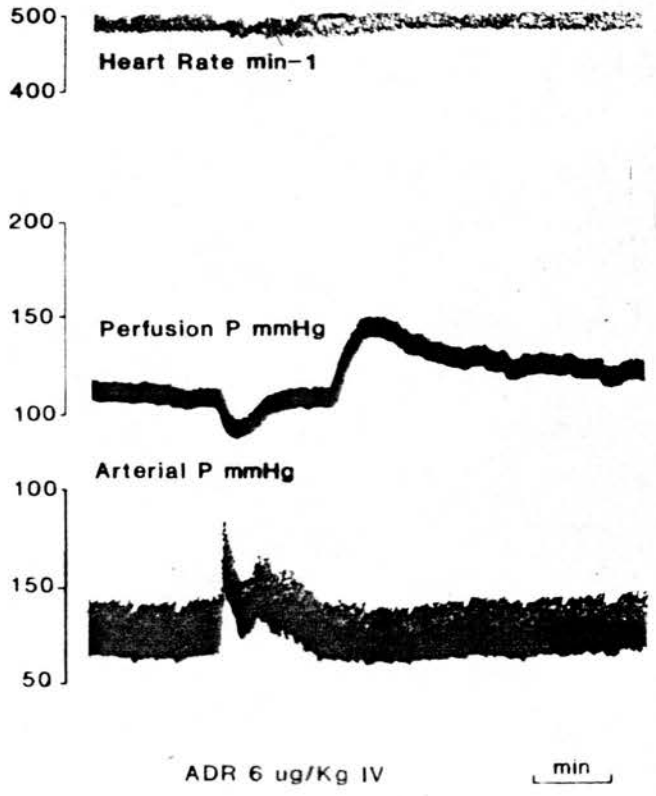

500
Heart Rate min-1
400
200
150
Perfusion P mmHg
100
Arterial P mmHg
100
150
50
ADR 6 ug/Kg IV
min

Fig. 3.3 g,h
The "Delayed" Hindlimb Perfusion With A Variety Of Stimuli.

Fig. 3.3 g
Glyceryltrinitrate IV And Asphyxia.
Perfusion pressure does not follow arterial pressure after GTN IV but does in response to asphyxia.

Fig. 3.3 h
Adrenaline IV.
Perfusion pressure falls as arterial pressure rises in response to ADR. After a delay perfusion pressure increases.

2) Response to IV clonidine Fig. 3.4 a,b,c.

In all cases arterial pressure rose briefly following IV clonidine. Heart rate fell as did perfusion pressure. The delayed response was always pressor with its magnitude reflecting the dose of clonidine. Comparison of the drop in perfusion pressure at 1.5 and nine minutes shows the former to be greater, though the final pressure was lower than the predose level. Clonidine acutely reduces perfusion pressure by in the first instance a neural action on vasomotor tone. This is more than the reflex reductions in peripheral resistance seen with phenylephrine and adrenaline as it outlasts the increase in arterial pressure which is brief and small compared to the responses to phenylephrine and adrenaline. The prolongation of the dilator effect beyond the duration of the arterial pressor action shows that this is not merely a reflex effect but a neurally mediated action of clonidine.

The direct action on the vasculature is pressor. The final fall in perfusion pressure is smaller than at 1.5 mins. The pressor effect in the perfused vasculature is of much longer duration than the arterial action and of comparable magnitude. The explanation offered for the prolongation of the action of phenylephrine in the periphery is insufficient to explain the discrepancy in magnitudes but accepting that clonidine is reducing vasomotor tone in excess of that associated with a pressor action helps. The arterial pressor action is overpowered by the accompanying reduction in vasomotor tone whilst still existing at plasma concentrations mediating vasoconstrtction. In the hindlimb vasomotor tone has fallen when clonidine arrives and does not attenuate the pressor effect, prolongation arises from diffusion within the delay tubing and lack of attenuation by falling vasomotor tone. That the overall peripheral action (compare 1.5 mins with 9 mins) is not dilator argues that an action at presynaptic nerve terminals reducing transmitter release is unimportant. How important a contribution this action makes cannot be derived from this experiment. Accepting the argument for an arterial pressor action of short duration makes part of the reduction in hindlimb resistance a reflex effect. Over a few minutes plasma levels of clonidine fall and likewise the suppressed pressor action. Vasomotor tone would rise a little in

Fig. 3.4 a,b

IV Clonidine And The "Delayed" Hindlimb Perfusion

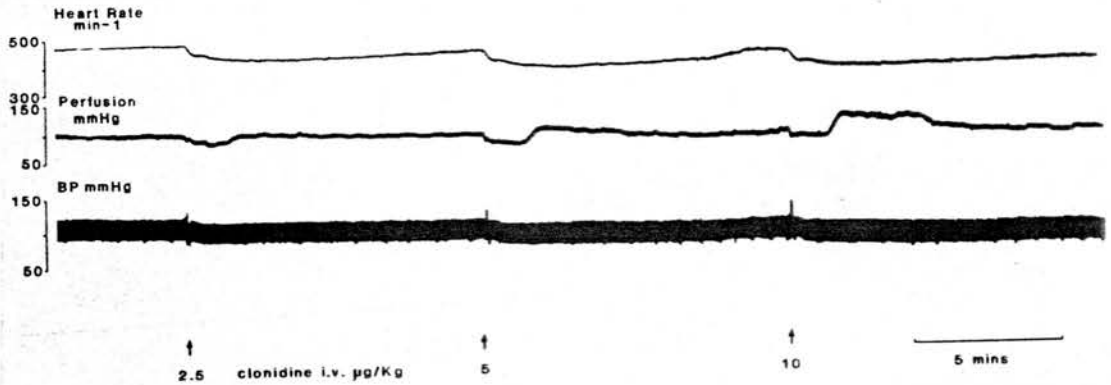


Fig. 3.4 a

Three Applications Of Clonidine IV

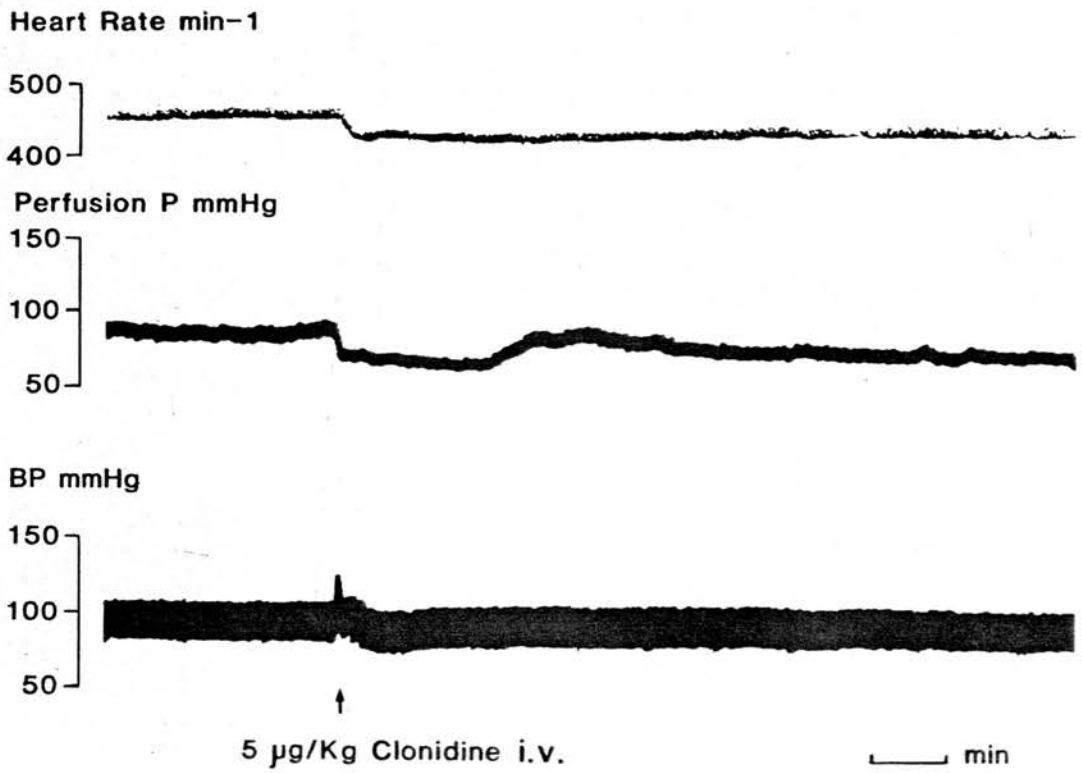
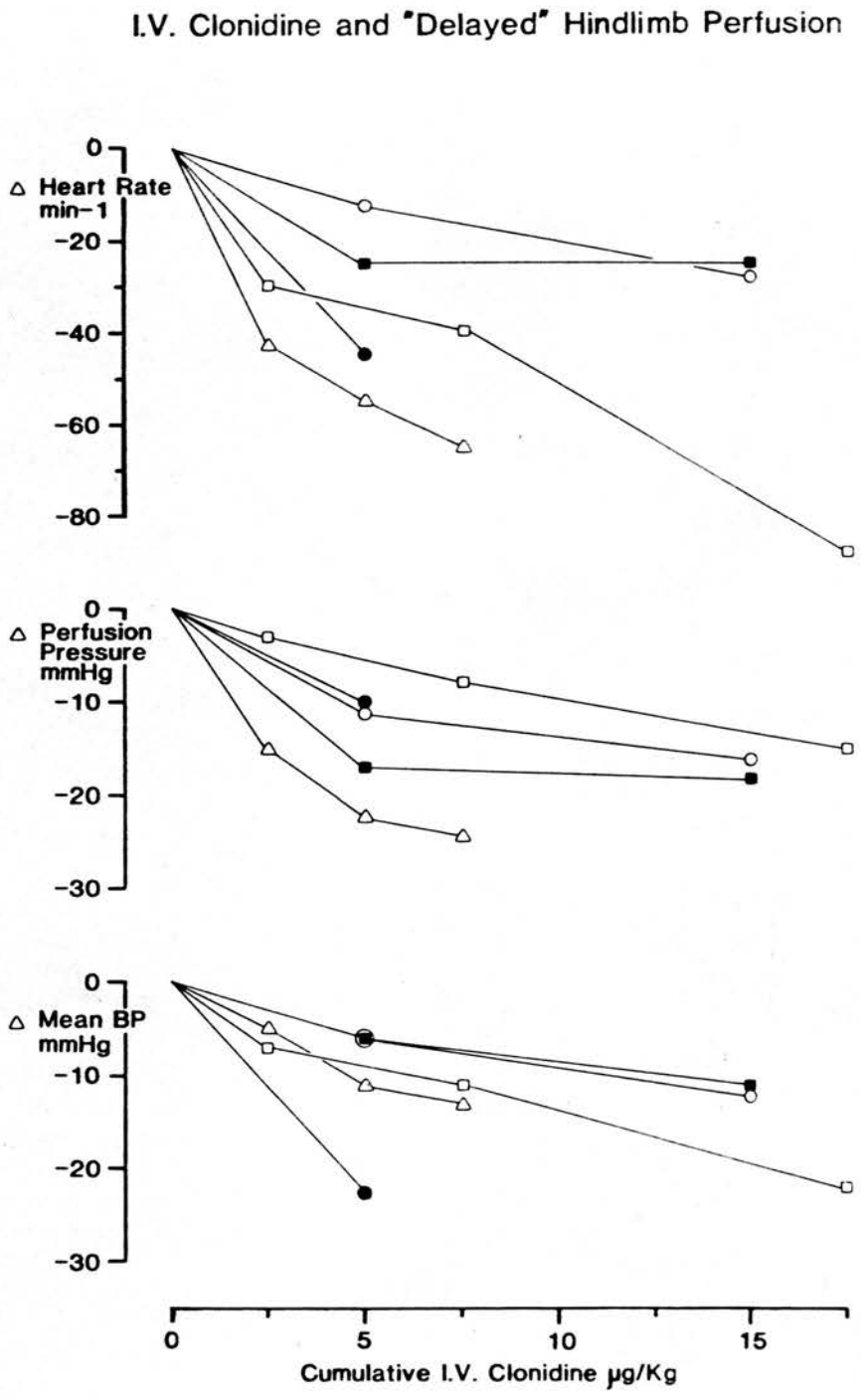


Fig. 3.4 b

One Application Of Clonidine IV

Fig. 3.4 c

IV Clonidine And The Delayed Hindlimb Perfusion.



Compilation of results from five experiments.

The responses are measured 9.5 minutes after each administration.

recognition of this. Therefore when comparing 1.5 with 9 mins after IV clonidine vasomotor tone is likely to have risen and the overall peripheral action of clonidine cannot be taken to be the difference.

Conclusions

A novel preparation for viewing the acute action on hindlimb vasomotor tone has been developed and, by showing predictable responses to known vasoactive agents, has been validated.

Clonidine was shown to cause an immediate fall in vasomotor tone and when the net effect of peripheral and central actions is viewed, at nine minutes after IV injection, an overall reduction in vascular resistance is seen. The magnitude of each action cannot be determined with this preparation. This would require a cross perfusion of the vasculature, administration of clonidine to the donor animal would allow direct vasoconstrictor and presynaptic actions to appear in the hindlimb and, assuming no change is vasomotor tone, their magnitude could be compared. Similarly clonidine given to the animal with the perfused hindlimb would allow only actions on vasomotor tone to appear.

The Central Administration Of Clonidine

A method of investigating the site of a drugs action is by selective administration to prospective areas. Regardless of how achieved the concentration at the site of action will determine both the intensity and duration of any response. IV administration (chapter 2) shows that the response to clonidine is very rapid, implying easy access to the site of action from the blood stream and that the pharmacological effects appear rapidly.

In the rat the cerebral circulation is derived from the two internal carotid arteries and the two vertebral arteries which supply the Circle of Willis through the basilar artery. The origins of the right and left subclavian arteries differ, the left arises from the arch of the aorta whilst the right originates from the common carotid artery. This asymmetry is important in central drug administration. The vertebrals also give rise to the ventral spinal artery which runs caudally down the ventral ssurface of spinal cord. The ventral spinal artery is an anastomotic chain formed by a series of ventral radicular arteries that reach the spinal cord along the spinal nerves. Reports on the importance of the vertebral arteries in the rat vary. Wellens et al (1976) suggest that the vertebrals in the rat play no part in supplying blood to any part of the brain. This study is based upon microsphere, methacrylate infusions and IVert administration of alpha-methyldopa. Methacrylate and microspheres when injected IVert only appeared in the muscle surrounding the neck. Alpha methyldopa did not show an enhanced action on blood pressure and heart rate after IVert administration. The methacrylate study aside the methods and conclusions appear reasonable. Mitchell & Himwich (1966) do show a role for the vertebrals in maintaining cerebral blood flow and Haywood et al (1980), using injection of biological black dye, show that the vertebrals supply the brainstem.

Experiments

Administration Of Clonidine By The Following Routes:-

1) Into the internal carotid artery.
2) Into the lateral cerebral ventricle.
3) Into the vertebral artery.

Methods

1) Intracarotid Clonidine. Administration was accomplished by inserting a cannula into the external carotid and pushing it to the bifurcation formed by the internal and external carotid. This necessitated the removal of the hyoid bone. To restrict the distribution of clonidine to the internal carotid artery the occipital and superior thyroid arteries were ligated. Clonidine was injected against the flow of blood over 40 seconds in 40ul with a 50ul syringe. An alternative approach could involve double cannulation of the the common carotid, allowing injection into the carotid, possibly combined with ligation of the occipital, external carotid and superior thyroid arteries. The ligations would be optional and would serve to limit drug distribution. Another approach is to insert a cannula down the right brachial artery to the common carotid artery, after side branches are tied off injected drug passes up the right common carotid.

2) Intraventricular Clonidine. A 26 gauge needle was inserted into the lateral ventricle using a stereotaxic frame and anchored with dental cement to a screw attached to the skull. Injections were given in 10ul over 60 seconds. Clonidine was made up in saline and tests with saline alone showed no cardiovascular changes accompanying injection. Evans blue dye was injected into the ICV cannula at the end of the experiment and distribution of dye throughout the ventricular system was taken as establishing the correct location of the cannula. Once the technique was mastered all injections were into the lateral ventricle system and Evans blue found throughout the ventricular system.

Coordinates from the Bregma 0,0,0	caudal	0.5 mm
	lateral	1.3 mm
	depth	5.0 mm

3) Intravertebral Clonidine. Intravertebral administration is commonly used in cats and dogs but rarely in rats. Haywood et al (1980) placed a cannula in the left brachial artery with its tip at the point of separation of the vertebral artery from the subclavian,

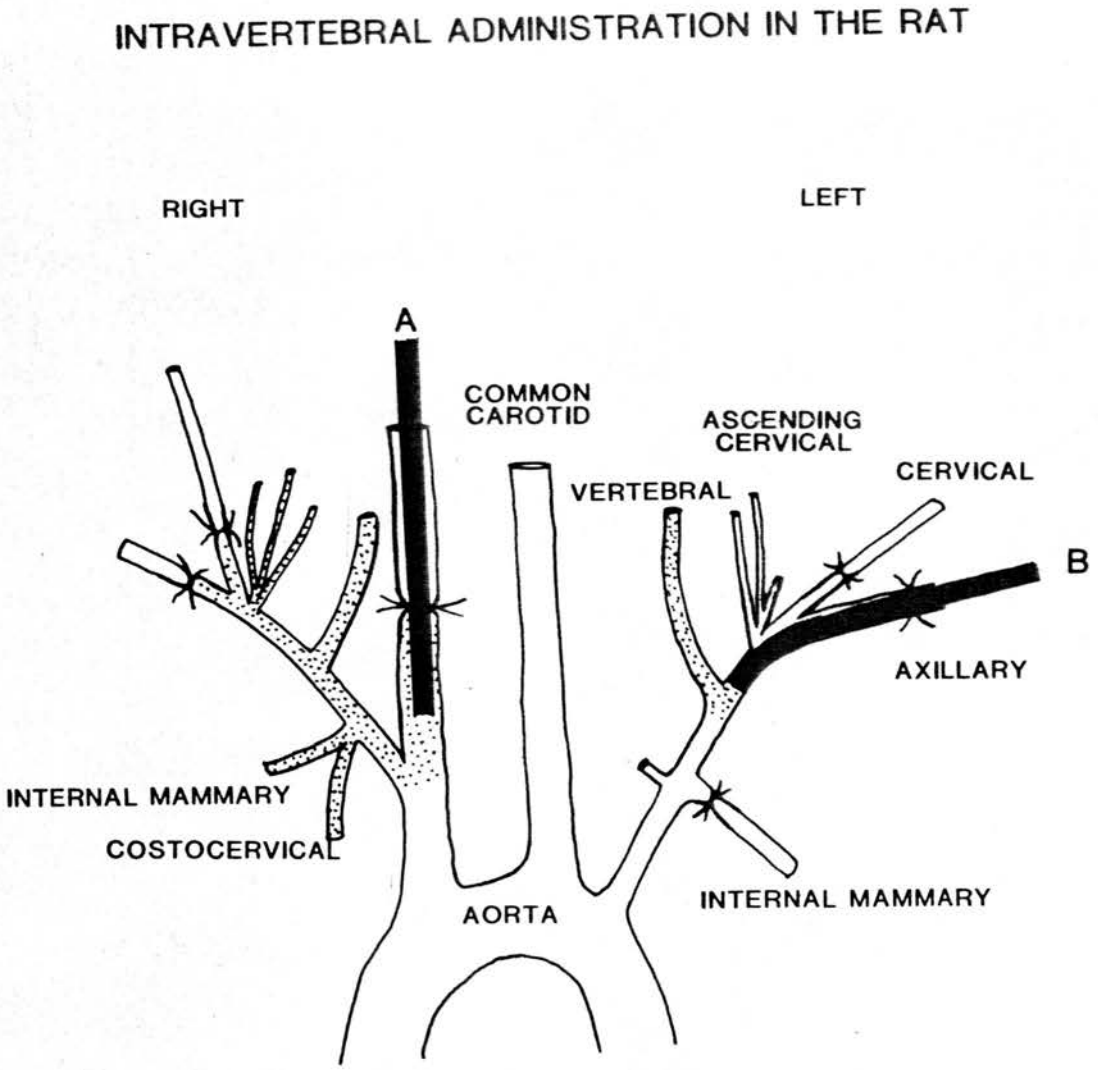


Fig. 4.1

Techniques for intravertebral administration of drugs.

Two methods are shown, A that used by the experimenter and B that more commonly used in cats and used in the rat by Haywood et al (1980). Solid black indicates the cannula and the stipled areas the likely pattern of drug distribution.

The asymmetry in the carotid/subclavian arteries is important in technique A but not in B.

in an accompanying diagram the cervical and mammary arteries were shown as being ligated but no mention of the latter is made in the text. The technique relies upon accurate placing of the cannula tip. If this is too deep and the injectate will enter the internal mammmary or costocervical arteries, if too shallow and the vertebral will be missed by the injectate in favour of any unligated arteries. Details of the surgery were not given. Attempts to utilize this approach involved the removal of the sternum and large amounts of muscle. Though Haywood et (1980) used their technique in recovery experiments. In practice this approach proved difficult surgically especially when trying to find the mammary artery whilst avoiding fragile veins. An alternative, see fig 4.1 A, was developed. A cannula is inserted into the right common carotid artery and all readily accessible branches of the right subclavian artery ligated, the axillary, ascending cervical and cervical. The cervical arteries are approached through the neck and a curved pair of watch makers forceps used to loop a tie around them. The axillary artery is approached as it emerges around the pectoral muscles, some care has to be taken not to rupture the axillary vein or damage the adjacent nerves, the 7th and 8th cervical and 1st thoracic.

The approach adopted has advantages over that used by Haywood et al (1980), the surgery is relatively easy and the exact location of the cannula tip is not as critical. The main disadvantage is that the distribution of injected drugs is not confined to the vertebral artery and will of necessity involve the costocervical and internal mammary arteries. However failure to adequately ligate one of the branches of the subclavian artery does not undermine an experiment, it only increases the spread of drug. Greene (1935) indicates that the vertebral may arise from the cervical trunk, this would negate the Haywood et al (1980) approach.

Results

1) Intracarotid Injection Of Clonidine (IC).

See figs 4.2, 4.3 and tables 4.T4-5. Injection of saline had no effect on blood pressure or heart rate. In the two figures control animals have undergone the same surgery as those given clonidine IC but received the clonidine IV. Comparison of the control and IC

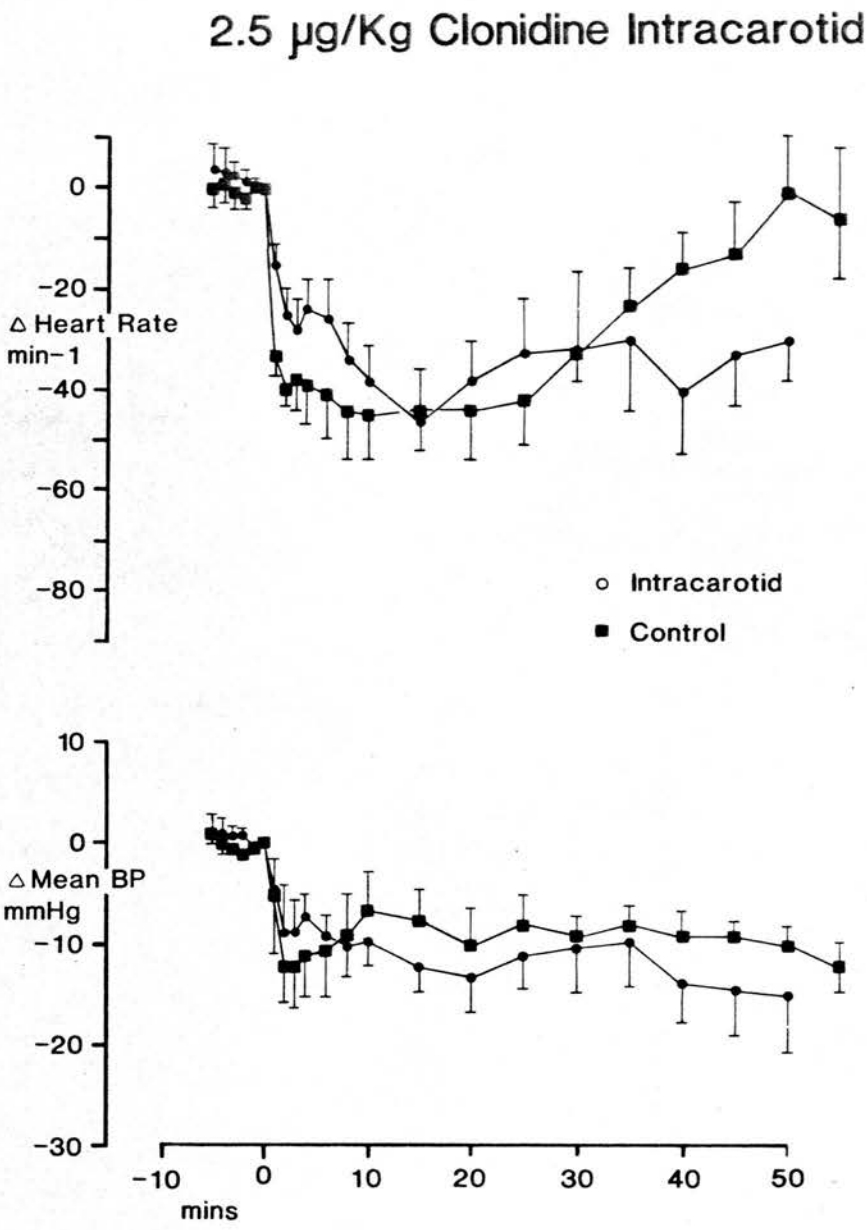


Fig. 4.2

IC Clonidine 2.5 ug/Kg, Comparison With Control 2.5 ug/Kg.

There are no appreciable differences in the blood pressure and heart rate responses.

The control has undergone the same surgery as the IC and clonidine is administered IV.

Control N=5, IC N=5

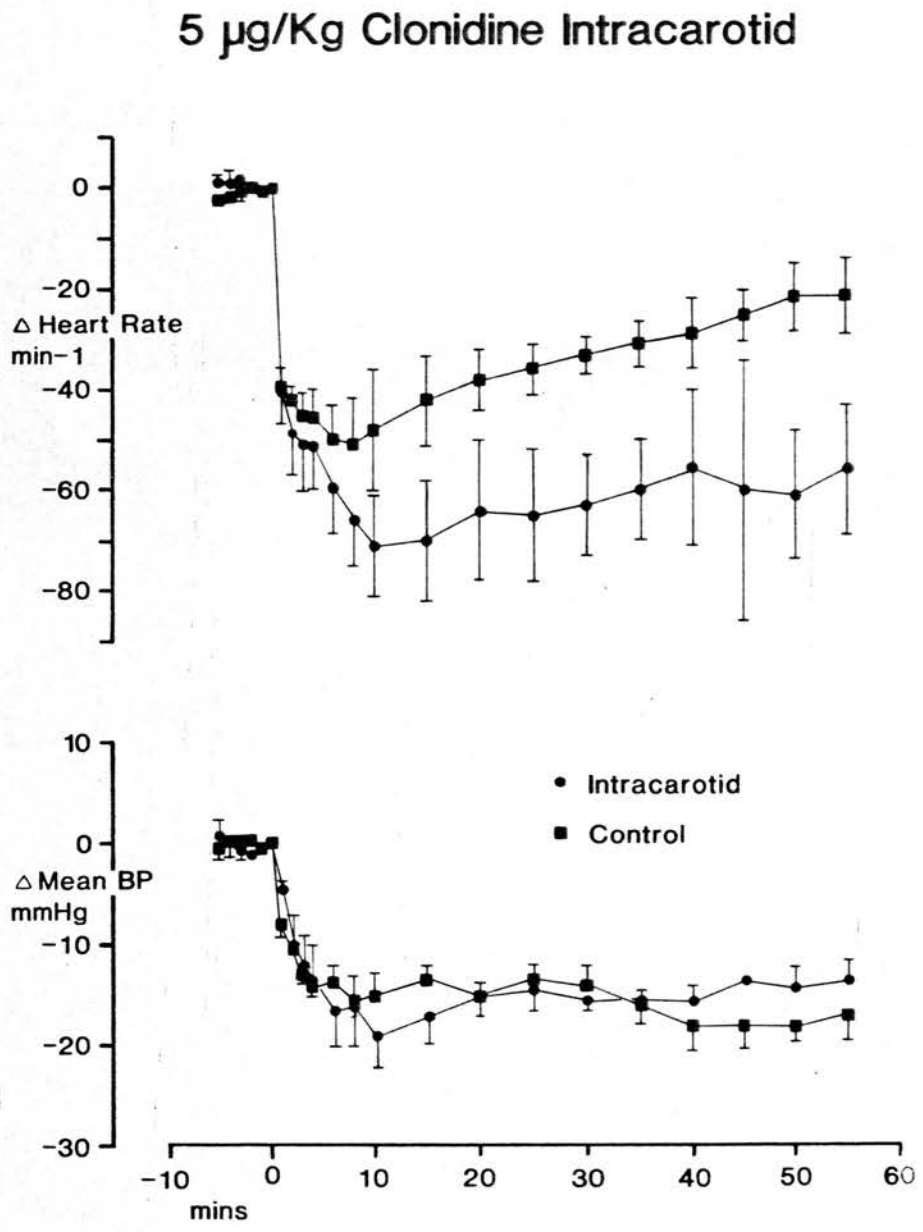


Fig. 4.3

IC Clonidine 5.0 ug/Kg, Comparison With Control 5.0 ug/Kg.

The blood pressure responses are very similar however the IC group display a greater fall in heart rate than the control.

The control group received clonidine IV.

Control N=4, IC N=4

animals shows no difference in the arterial blood pressure responses. However, at 5 ug/Kg a greater fall in heart rate is seen, with 2.5 ug/Kg this difference is not found.

2) Intraventricular Clonidine (ICV).

See figs 4.4, 4.5, 4.6, 4.7 and tables 4.T6-8. The fall in blood pressure is dose dependent over the range studied, 1-10 ug/Kg. Heart rate changes have not been included in fig 4.4 as ratemeters were not available in all experiments. ICV administered clonidine leads to a much reduced onset of action compared to IV administration. Comparison of the dose response curve with that following IV injection (see chapter 2) shows no difference in peak effects but comparison at a dose of 5 ug/Kg between ICV and animals prepared for ICV but injected IV shows the former to be more potent, a surprising result which suggests that the surgery involved in placing the ICV cannula alters the magnitude of the drug response.

Although the response is dose dependent the time course is similar over the range of doses used. The only aberration is the slight pressor effect seen during the 2 mins after the 1 ug/Kg injection.

3) Intravertebral Clonidine Administration (IVert).

See figs 4.8, 4.9, 4.10a, 4.10b and tables 4.T1-3. 0.25 ug/Kg of clonidine lowers blood pressure and heart rate when adminstered into the vertebral artery. Saline similarly administerd has minimal effects and the quantity of clonidine used has only a small hypotensive action when administered intravenously. This is clearly shown in a number of studies where clonidine was alternately injected IV and IVert.

The recovery from a single IVert application of clonidine was followed in a number of animals, it is faster than that seen after a equipotent IV application but is by no means dramatic.

Discussion

The major differences appeared in the response to clonidine when administered by one of four routes, IV, IVert, ICV and IC. The differences involved the magnitude of response and speed of onset.

ICV administration appears more potent than IV injection but

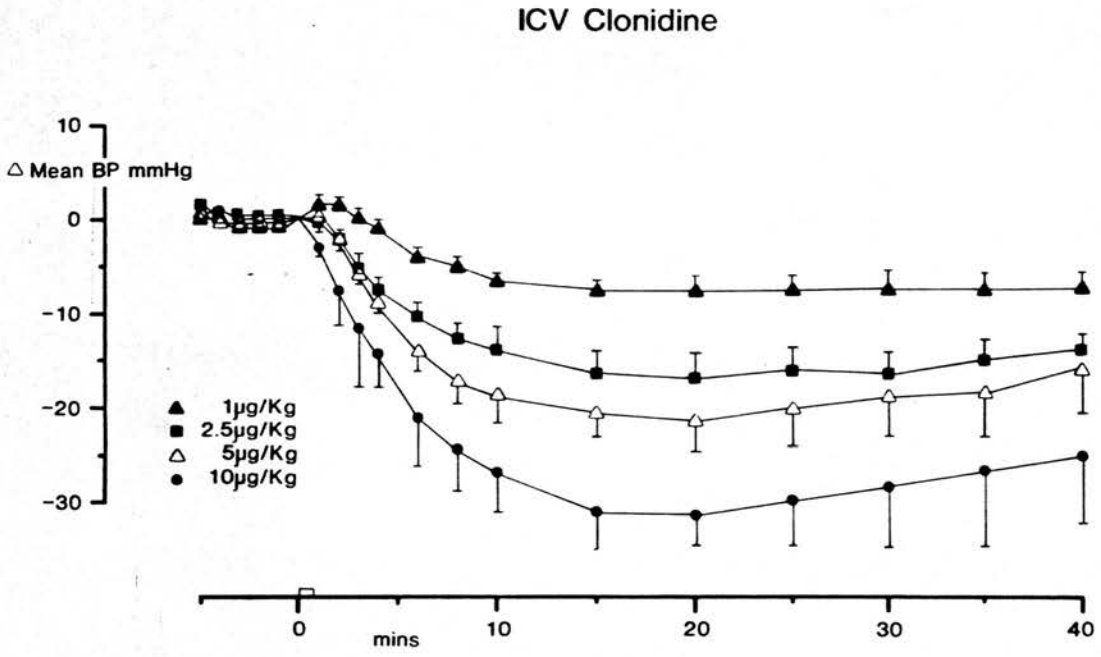


Fig. 4.4

ICV Clonidine 1, 2.5, 5, 10 ug/Kg.

Only blood pressure responses are shown as heart ratemeters were not available for all the experiments.

The onset of action is slow, peaking between fifteen and twenty minutes.

The response to ICV saline is not shown but is minimal.

1 ug/Kg N=4, 2.5 ug/Kg N=4, 5 ug/Kg N=7, 10 ug/Kg N=4

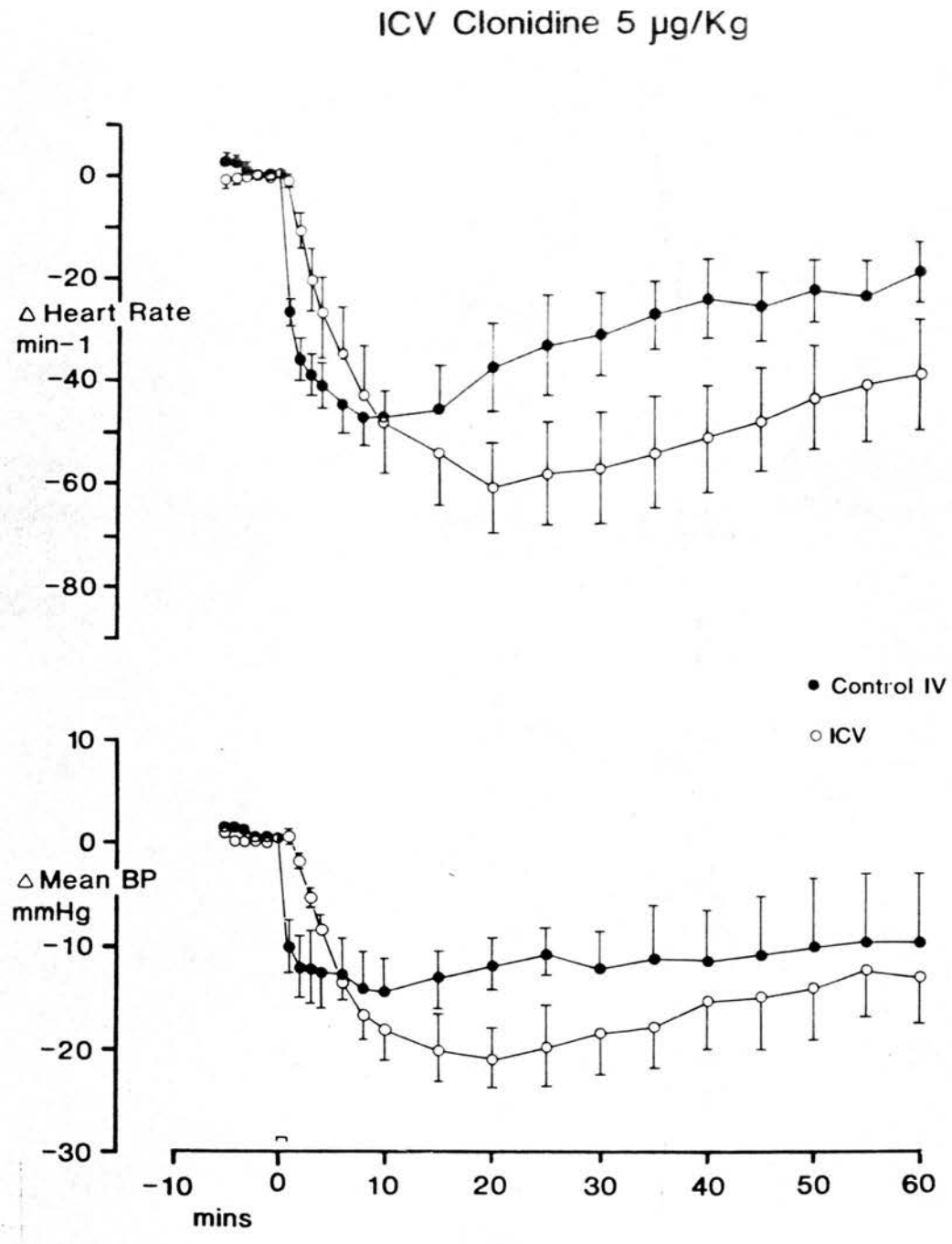


Fig. 4.5

ICV Clonidine 5 ug/Kg And Control IV 5 ug/Kg.

Control has an ICV cannula implanted but has clonidine given IV.

ICV is more potent but the timecourse for each differs, see next graph. Fig. 4.6

The control group were underwent the same surgery as the ICV animals but were injected IV with clonidine.

ICV N=7, Control N=6

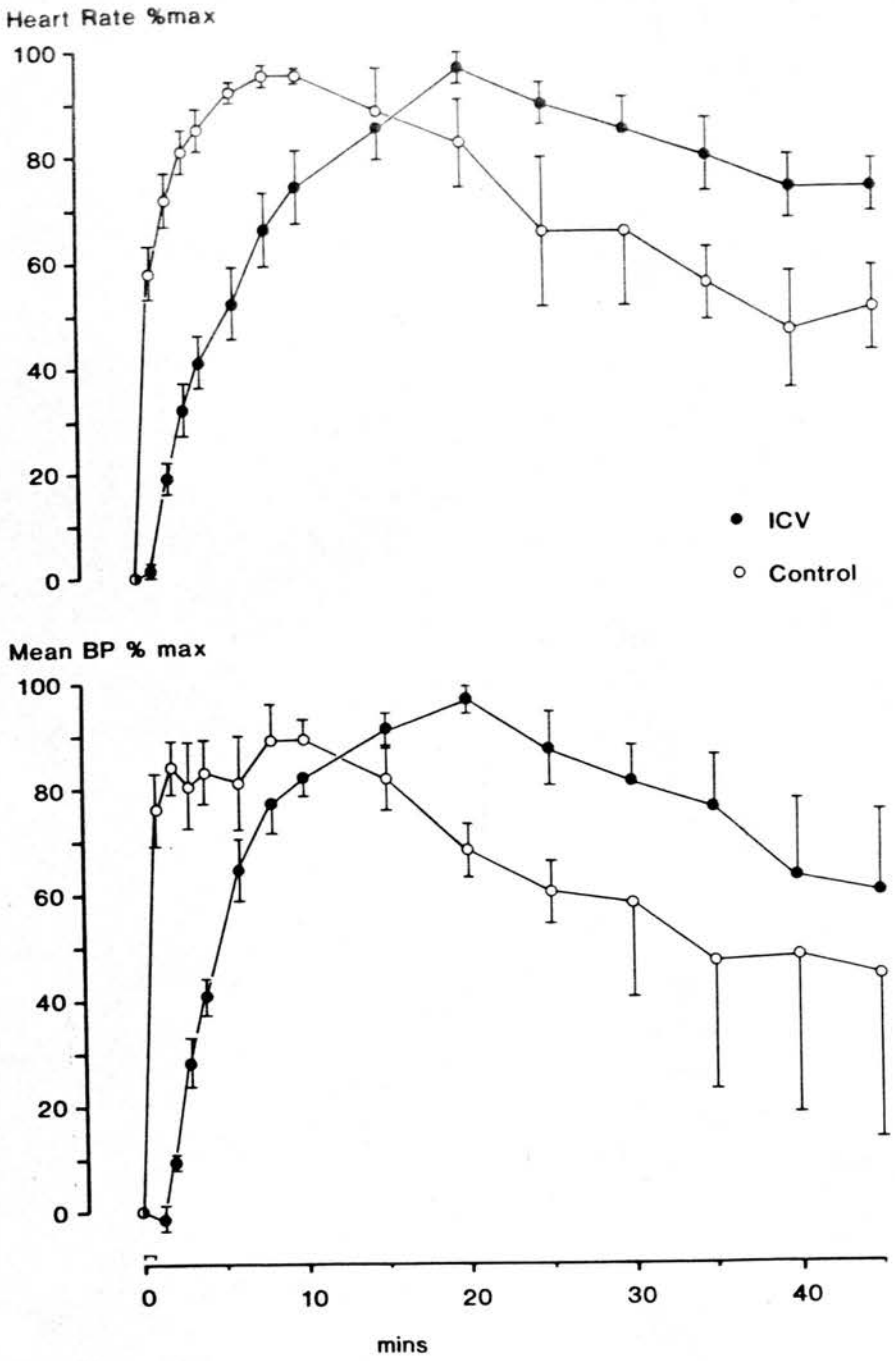


Fig. 4.6

ICV Clonidine and Control Timecourse.

Data on blood pressure and heart rate has been reasessed with respect to timecourse. For each animal the response at each time has been recalculated as % of maximum and this data used in plotting the graph. Clearly IV control animals achieve maximum falls in blood pressue and heart rate before animals given clonidine ICV.

The failure of graphs to reach 100% maximum arises because not all animals achieve their peak responses at the same time after dosing.

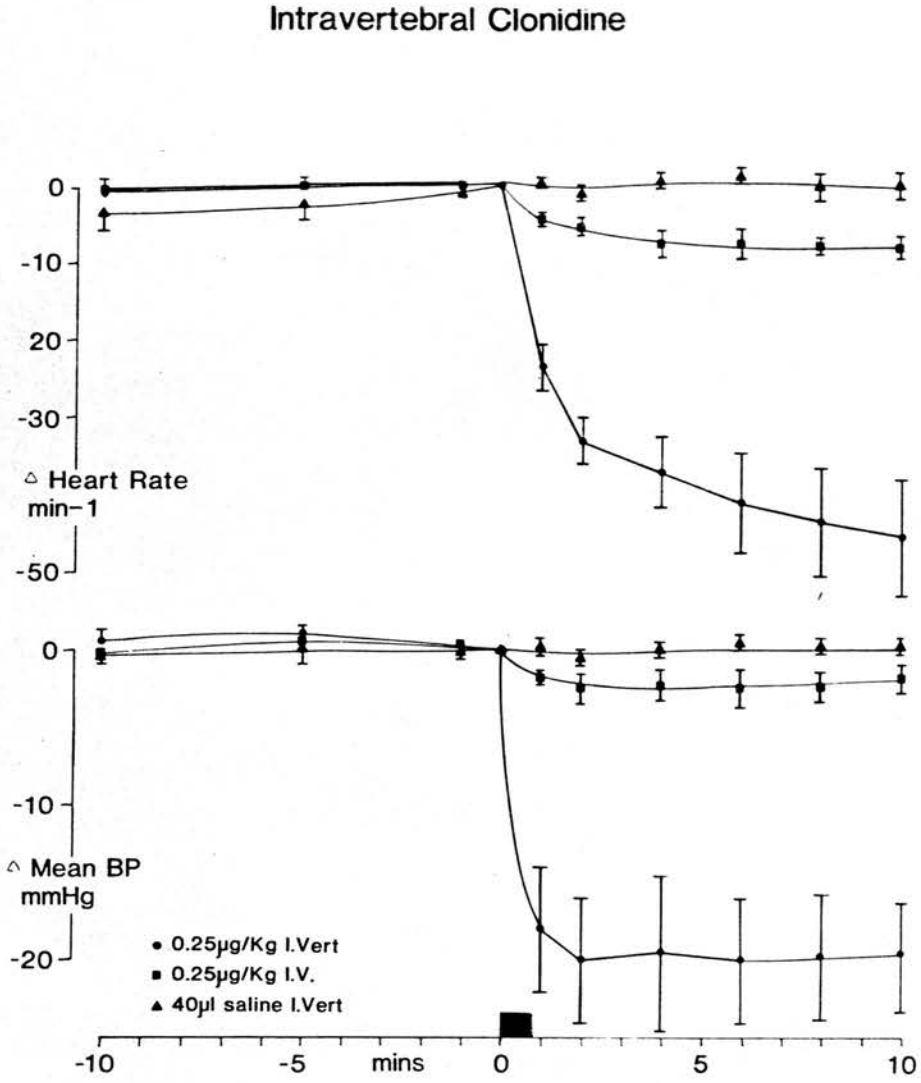


Fig. 4.7

IVert Clonidine Comparison With IVert Saline And IV Control

All animals underwent the same surgery.

The volume of saline is that used for the clonidine injection, and is given over the same 40 second period as was the IV dose.

IVert ClonidineN=5, IVert Saline N=8, IV Clonidine N=7

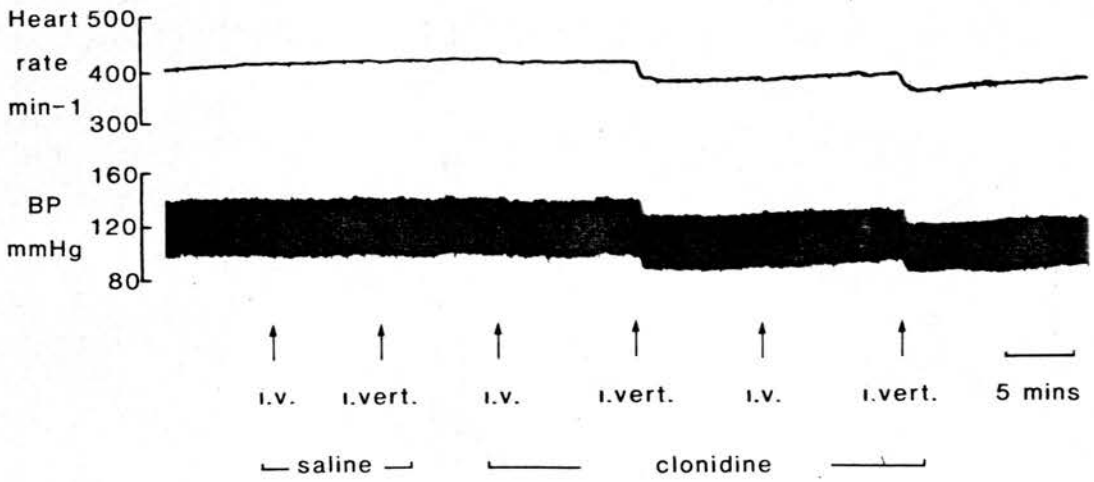


Fig. 4.8

IVert Clonidine Trace.

Recording of a single experiment where clonidine or saline were given IV or IVert. Volume used was 40 ul and the quantity of clonidine 0.25 ug\Kg. Doses given every 10 mins.

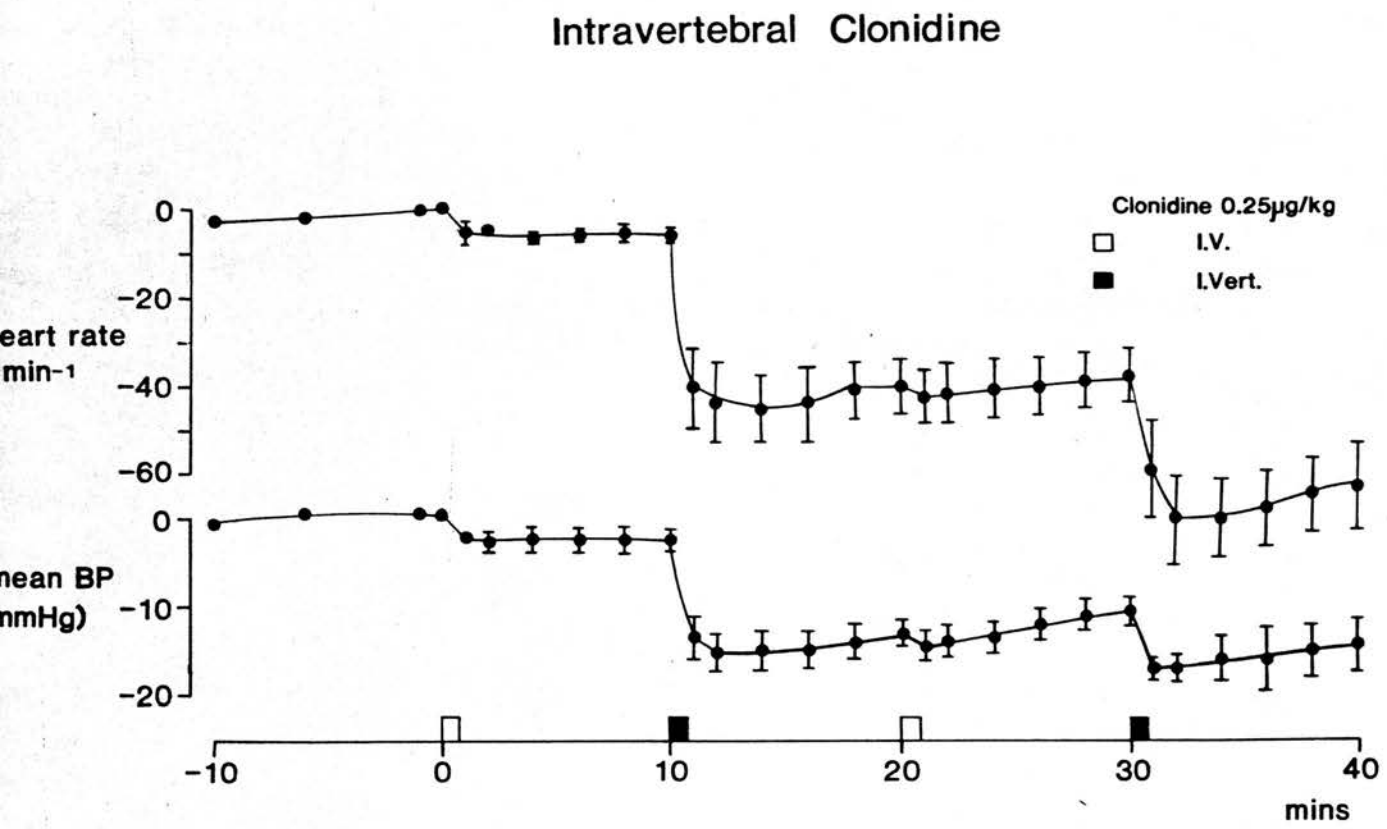


Fig. 4.9

Successive IV And IVert Clonidine

N=4

Fig. 4.10

Longterm Response To IVert Clonidine Showing Recovery.

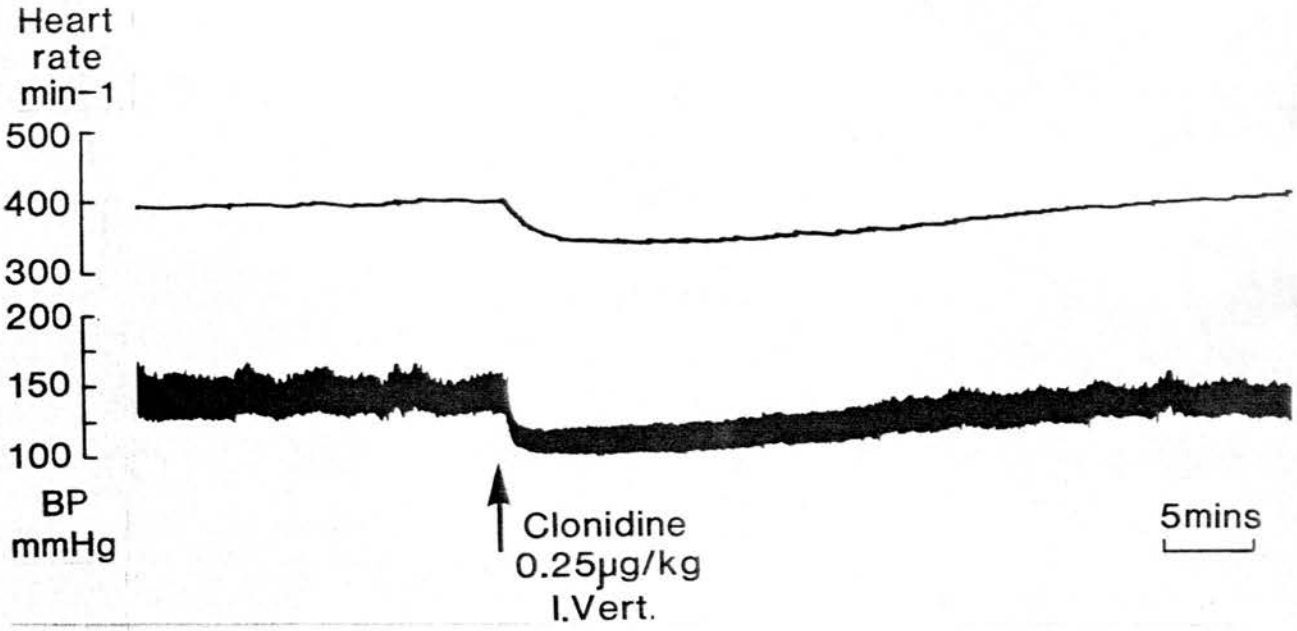


Fig. 4.10a

Blood pressure and heart rate trace from one experiment

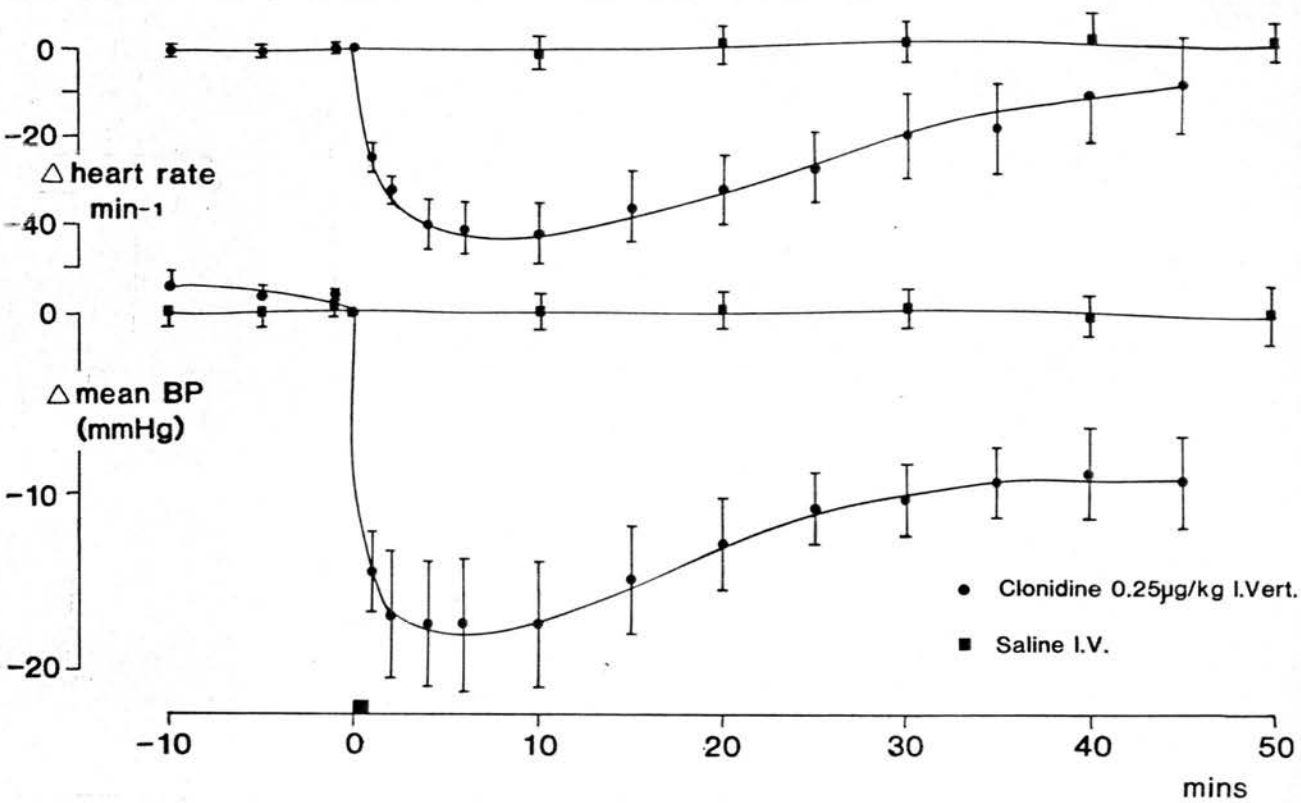


Fig. 4.10b

IVert clonidine, graph showing recovery and comparison with saline IV. N=6

has a severly retarded onset suggesting that the site of action is reached only with difficulty. Though more potent the increase does not match that seen with injection into the ventricular system in dogs, monkeys and cats where potency greatly increased (Bolme et al 1975) (Schmitt & Schmitt 1968). The abscence of greatly enhanced potency has been previously noted in the rat (Borkowski et al 1975) (Finch et al 1977).

That onset is progressive not delayed suggests that the site is easily reached by a portion of the dose. But if action is at a single site, which a portion of the dose can reach rapidly, it becomes hard to explain why the remainder fails to act producing a rapid supramaximal response. A second site of action that is less easily attained could be reasonably envisaged.

Following ICV administration a quantity of clonidine, capable of reducing blood pressure and heart rate when injected intravenously, is deposited in the ventricular system and judging by the pattern seen with Evans Blue spreads widely. This not withstanding the likely initial volume of distribution is very limited and a much higher concentration within CSF than seen after IV administration would be anticipated. The importance of this to the subsequent cardiovascular changes is not shown in this series of experiments.

IC administration is no more potent than IV injection in reducing heart rate and blood pressure. This appears to be the first study undertaken on IC clonidine administration in the rat although the lack of hypotensive potency has been noted in larger animals (Constantine & McShane 1968) (Laubie et al 1976). A direct action on baroreceptors, increasing their sensitivity, leading to a fall in blood pressure may be discounted in the rat, although reported in other animals. The route of administration would be expected to result in a high concentration of clonidine around at least one set of carotid body baroreceptors and any direct actions would be expected to appear. In this context the greater fall in heart rate, seen with 5 ug/Kg IC, may be of importance although the variations between animals were large. It is possible that the surgery involved in the chosen method of intracarotid administration may have damaged the carotid sinus baroreceptors.

Intravertebral clonidine is very effective in lowering blood

pressure and heart rate. This indicates that the new method used to inject into the vertebral artery enables clonidine to reach an area involved in blood pressure control. The technique is easy to utilize requiring about 10 minutes surgery. If time was very pressing ligation of the branches of the subclavian artery could be ommitted finally requiring no more surgery than carotid cannulation. In these studies the branches were ligated when found. This technique could permit the widespread use of intravertebral injection in the rat, presently an under-used approach. The use of intravertebral injection in the cat and dog is common and in most cases nothing is discovered using a large expensive animal than could not be shown in the rat.

Intravertebral administration of clonidine is the most effective route of the four used in the rat. This appears to be the first study of intravertebral clonidine in the rat although a number of studies have been undertaken in dogs and cats, noting the hypotensive potency (Sattler & Van Zwieten 1967) (Katic et al 1972) (Constantine & McShane 1968). Given the potency of this method of administration only one dose was studied in detail, 0.25 ug/Kg. This dose when given IV to rats prepared for IVert administration has almost no action on heart rate or arterial blood pressure. This means that the the drug induced response is only due to first pass uptake into areas supplied with blood from the vertebral artery. That this is the case is not surprising given clonidine`s lipophilic properties, what is surprising is the persistance of the fall in heart rate and blood pressure, figs 4.10a and 4.10b. The fall in blood pressure resulting from 0.25 ug/Kg IVert is similar to that caused by 5 ug/Kg (chapter 1) a factor of X20. The return to predose blood pressure is faster with IVert administration and must represent the washing out of clonidine from the brain by blood containing a low level of drug, a measure of clonidine's ability to move out of the brain. Clearly the drug crosses the blood brain barrier readily when moving from blood to brain but movement in the other direction is poor which is strange given the concentration gradient which may be assumed to exist facilitating net movement.

These results clearly suggest that in the rat the vertebral arteries deliver blood to the brain, but do not indicate which areas are supplied. They contradict the conclusions drawn by Wellens et al

(1976), that the vertebral arteries are not involved in the cerebral blood supplly. The size of rat used differs markedly, Wellens et al (1976) used 400-500 gm whilst all the rats used with IVert clonidine were less than 200 gms, suggesting an age difference. Alternatively, Greene (1935), the lability of the circle of Willis in the rat might reconcile the data.

Given that the quantity of clonidine used when applied intravenously had only minor effects on the cardiovascular system it follows that the peak concentration of clonidine appears in the brain immediately after the end of the slow IVert infusion. The dose used had no pressor actions, IV adminstration of the same quantity did not increase arterial pressure, therefore clonidine not passing into the brain on the first pass is unlikely to show any peripheral vasoconstrictor effects. Thus the onset of the cardiovascular effects is uncomplicated by baroreflex mediated responses to a pressor effect and the rate of onset of the response depends solely upon the hypotensive properties of the drug. The peak reduction in arterial pressure appears at 10 minutes not at the end of the infusion and the reduction in heart rate is also much greater after ten minutes than at the end of the infusion. This indicates that the mechanism of the cardiovascular response to clonidine in the rat has a temporal component, possibly representing actions at two or more different sites.

Tables for Chapter 4

Animals Prepared for IVert Administration

Blood Pressure mmHg

-10	-5	-1	0	1	2	4	6	10 Time
.....Clonidine 0.25 ug/Kg IV.....								min
-0.5	0	0	104.5	-1.5	-1.5	0	0	0.5
-0.5	0.5	0	114.5	-1	1.5	1.5	1.5	1.5
0.5	0.5	2	124	-3.5	-3.5	-2	-2	-2.5
0	0	0	119.5	-2	-5	-6.5	-7	-7
0	1	0	112	-2	-1	-1	-2	-1
-2.5	-2.5	0.5	134.5	0	-5.5	-4.5	-4.5	-1
0	0	0	87.5	1.5	-2	-3.5	-3.5	-2.5
.....Clonidine 0.25 ug/Kg IVert.....								
-2	-1.5	0.5	111.5	-8.5	-10	-10.5	-10.5	-9
1.5	1.5	0	126	-14	-15	-15	-16	-16.5
3	2	0	113	-26	-25	-24	-24	-22
0	2	1	136.5	-28	-33	-33	-33	-32
0	1	0.5	127.5	-15	-16.5	-16	-17	-18
.....Saline 40 ul IV.....								
-1.5	5.5	-0.5	127.5	2.5	1.5	-1.5	1.5	-1.5
0	0	0	110	0	0	0	0	0
1.5	1	-1	118.5	0	-0.5	-1.5	-0.5	-0.5
-1	-1	0	127	0	0	0	0	1
1.5	0	0.5	114.5	-0.5	-0.5	-0.5	-0.5	1.5
-1	-1	0	123.5	0	-1.5	2	2	0.5
-1.5	-2.5	-3	139	-0.5	-3	-0.5	-1.5	-2.5
-3	-1.5	0.5	117	0.5	-1	2.5	2.5	2.5

Heart Rate min-1

.....Clonidine 0.25 ug/Kg IV.....								
-2.5	-5	0	375	-5	-5	-5	-2.5	-2.5
0	0	0	393	-5	-7.5	-7.5	-7.5	-10
-5	0	0	400	-6	-7.5	-7.5	-7.5	-7.5
0	0	0	368	-5	-7.5	-10	-10	-10
0	0	0	413	0	0	0	0	-2.5
2.5	2.5	0	423	-5	-5	-12.5	-12.5	-12.5
2.5	2.5	0	383	-2.5	-5	-12.5	-12.5	-12.5
.....Clonidine 0.25 ug/Kg IVert.....								
0	2.5	0	413	-32.5	-37.7	-37.5	-35	-32
-2.5	-2.5	0	390	-20	-22.5	-22.5	-25	-22.5
0	-2.5	-2.5	410	-27.5	-32.5	-32.5	-32.5	-60
-2.5	2.5	0	400	-22.5	-40	-50	-55	-55
-3.5	0	0	413	-15	-32.5	-42.5	-57.5	-57.5
.....Control Saline 40 ul IV.....								
-10	-15	0	388	0	0	-2.5	2.5	2.5
-2.5	-2.5	0	415	2.5	2.5	2.5	5	2.5
0	0	0	343	0	-2.5	-2.5	-2.5	-2.5
7.5	2.5	2.5	420	-2.5	-5	-2.5	-2.5	-10
2.5	2.5	0	393	0	0	0	0	0
-7.5	-2.5	0	395	0	0	5	5	5
-5	-2.5	-2.5	403	-2.5	-5	0	0	-2.5
-12.5	-5	-2.5	363	0	2.5	5	5	5

The rats were prepared for both IV and IVert adminstration and were given IVert clonidine (0.25 ug/g), IVert saline (40 ul) or IV clonidine (0.25 ug/Kg) and the response followed for ten minutes.

IVert Longterm

time	.....Heart Rate.....					
min			min-1			
-10	1	-2.5	2.5	2.5	-3.5	-2.5
-5	2.5	-3.5	0	2.5	0	-5
-1	0	0	2.5	0	0	0
0	412	390	350	400	413	415
1	-32.5	-20	-30	-22.5	-15	-27.5
2	-37.5	-22.5	-27.5	-40	-32.5	-32.5
4	-37.5	-22.5	-30	-55	-57.5	-37.5
6	-35	-25	-27.5	-55	-57.5	-45
10	-32.5	-22.5	-25	-55	-52.5	-60
15	-30	-10	-17.5	-50	-52.5	-55
20	-35	5	-10	-30	-55	-25
25	-35	5	-10	-30	-55	-35
30	-35	10	2.5	-17.5	-52.5	-25
35	-35	15	5	-7.5	-45	-40
40	-37.5	20	15	0	-42.5	-17.5
45	-37.5	22.5	15	10	-40	-20

	.....Blood Pressure.....					
			mmHg			
-10	-1	1.5	-2	2.5	2	5
-5	-0.5	3	0	2	1	0
-1	0.5	0	-2	1	0.5	0
0	111.5	126	103	136.5	127.5	135
1	-8.5	-14	-10	-24	-15	-15
2	-10	-15	-8	-33	-16.5	-19
4	-10.5	-15	-8	-32.5	-16	-23
6	-9.5	-16	-8	-32	-16	-25
10	-9	-16.5	-7	-26	-18.5	-27
15	-6.5	-15	-7	-18	-17.5	-27
20	-6.5	-12	-5	-22	-15.5	-17
25	-6.5	-13	-4	-12.5	-15	-15
30	-7	-13.5	-2	-10.5	-15	-15
35	-6	-13.5	-2	-9.5	-11.5	-15
40	-7.5	-13	1	-10.5	-9	-15
45	-7.5	-14	0	-13.5	-8	-15

The changes in blood pressure and heart rate were followed in these rats for at least forty five minutes after administration, rather than for only ten minutes.

Alternate IV IVert 0.25 ug/Kg Clonidine

	Time min	.....Heart Rate..... min-1				.....Blood Pressure..... mmHg			
	-10	-2.5	-5	-5	0	-0.5	0.5	-2	-0.5
	-5	-5	-2.5	0	0	-0.5	1	0	1.5
	-1	0	0	0	0	0	0	0	2
IV	0	375	418	400	367.5	104.5	113	119.5	124
	1	-5	-5	-6	-5	-1.5	-2	-2	-3.5
	2	-5	-5	-2.5	-7.5	-1.5	-1	-5	-3.5
	4	-5	-5	-7.5	-10	0	-1	=6.5	-2
	6	-2.5	-5	-7.5	-10	0	-2	-6.5	-2
	8	-2.5	-2.5	-7.5	-10	0.5	-1	-7	-3
IVert	0	-5	-2.5	-7.5	-10	1.5	-1	-7	-3
	1	-32.5	-40	-65	-25	-7.5	-12	-16	-18
	2	-35	-42.5	-70	-30	-10.5	-13	-18.5	-19
	4	-35	-45	-67.5	-35	-9.5	-13	-18	-19
	6	-32.5	-45	-67.5	-33.5	-10.5	-12	-18	-19
	8	-32.5	-40	-60	-32.5	-10	-11	-17	-17
IV	0	-32.5	-40	-57.5	-30	-9.5	-11	-16	-15
	1	-35	-45	-60	-32.5	-11.5	-11	-16.5	-18.5
	2	-30	-45	-60	-32.5	-11.5	-10	-16.5	-17.5
	4	-32.5	-40	-60	-32.5	-12	-9	-16.5	-16
	6	-35	-37	-60	-30	-11.5	-7	-15	-14
	8	-32.5	-35	-57.5	-30	-11.5	-6	-14	-11.5
IVert	0	-32.5	-35	-55	-30	-11.5	-6	-14	-10.5
	1	-47.5	-62.5	-87.5	-37.5	-15	-15	-19	-20
	2	-47.5	-67.5	-85	-42.5	-13.5	-15.5	-18.5	-20
	4	-42.5	-65	-82.5	-50	-10	-14	-21.5	-19
	6	-37.5	-57.5	-82.5	-50	-7.5	-14	-24	-18
	8	-30	-35	-75	-57.5	-7.5	-12	-22	-17.5
	10	-25	-55	-72.5	-60	-7.5	-12	-22	-16

The rats were prepared for both IV and IVert administration of clonidine. Clonidine was given initailly IV, then IVert, again IV and finally IVert again.

Intracarotid Clonidine (5 ug/Kg) on Heart Rate and Blood Pressure

Heart Rate

mins	IC				min-1	IV			
-5	7	1	-3	1		-3	-6	2	-3
-4	6	0	-2	1		-2	-5	0	-1
-3	5	0	-1	3		-2	-4	3	-1
-2	3	0	-2	0		-2	-2	1	0
-1	0	0	-1	0		0	-2	2	-1
0	390	474	404	410		330	380	400	411
1	-40	-50	-48	-22		-38	-28	-48	-44
2	-46	-67	-55	-26		-42	-32	-48	-46
3	-43	-72	-59	-30		-43	-32	-52	-51
4	-46	-78	-62	-35		-45	-30	-56	-51
6	-46	-84	-63	-45		-52	-30	-61	-56
8	-52	-94	-64	-55		-56	-25	-67	-56
10	-58	-102	-61	-62		-58	-13	-67	-55
15	-68	-106	-53	-54		-58	-16	-53	-42
20	-60	-106	-44	-46		-43	-20	-48	-40
25	-52	-102	-47	-57		-48	-24	-31	-41
30	-58	-94	-48	-53		-35	-33	-24	-38
35	-62	-89	-46	-42		-37	-36	-34	-17
40	-68	-94	-31	-33		-44	-23	-38	-10
45	-68	-94	-39	-40		-40	-23	-23	-16
50	-68	-94	-37	-46		-40	-20	-22	-6
55	-54	-93	-37	-38		-40	-14	-26	-10

Blood Pressure

mmHg

-5	1	-3	5	1	2	-2	1	-2
-4	1	-3	4	0	1	0	1	-1
-3	-1	-2	3	-2	0	1	1	-1
-2	-2	-1.5	0	-1	0	2	0	-1
-1	-1	0	0	-1	0	-1	0	0
0	99.5	109	101	100	100	117	106	108
1	-4.4	-6	-4	-3	-5.5	-6	-11	-10
2	-18	-11.5	-5	-6	-10	-11	-10	-11
3	-20	-13	-10	-6	-15	-13	-12	-12
4	-22	-16.5	-8	-8	-17	-12	-12	-14
6	-25.5	-21	-10	-11	-18.5	-12	-13	-11
8	-26.5	-20	-10	-9	-22	-12	-15	-13
10	-26	-23	-11	-17	-21	-10	-15	-13
15	-19	-22.5	-10	-17	-17	-10	-15	-12
20	-13.5	-21	-10	-15	-18	-13	-13	-15
25	-11	-19.5	-12	-15	-15	-15	-9	-15
30	-14.5	-17.5	-13	-8	-13	-19	-10	-15
35	-15	-18	-13	-17	-14	-21	-14	-14
40	-15	-19	-12	-16	-21	-24	-14	-14
45	-13	-12	-13	-13	-19	-24	-13	-17
50	-14	-12	-11	-20	-19	-22	-14	-16
55	-10	-13	-10	-18	-19	-19	-10	-18

All the rats were prepared for both IV and IC administration of clonidine, the tables headed IC denote those receiving clonidine by that route and the tables headed IV denote those given clonidine by the alternate route.

Intracarotid Clonidine (2.5 ug/Kg) on Heart Rate and Blood Pressure

	.....IV.....				....IC....					
Time min	Blood Pressure mmHg									
-5	1	3.5	1.5	-1	-1	-1	3	1	9	0
-4	1	2.5	1	-2	-2	0.5	2	2	9	0
-3	0	-1.5	1	-1	-1	1	0	2	7	0
-2	0.5	-2.5	0.5	-2	-1	2.5	1	0	5	2
-1	0	-1.5	0	-1	0	0	0	-1	2	0
0	86	84.5	84	85	99	88	101	110	120	94
1	-10	-19	-5.5	-6	-6	1.5	0	-7	-1	-16
2	-14	-25	-7	-6	-8	-0.5	-4	-13	-2	-24
3	-14	-27	-6.5	-5	-7	-0.5	-10	-15	-1	-17
4	-14	-25.5	-6.5	-7	-3	-2.5	-6	-12	-3	-12
6	-15	-25.5	-6.5	-8	3	-4	-11	-16	-5	-10
8	-15	-20.4	-6.5	-6	4	-3	-15	-18	-6	-7
10	-15.5	-11	-5.5	-8	7	-6	-13	-17	-5	-6
15	-13	-13.5	-7	-9	4	-7	-15	-20	-6	-13
20	-9	-11.5	-8.5	-22	2	-2.5	-17	-20	-7	-18
25	-9	-13.5	-3.5	-14	2	1.5	-16	-15	-10	-15
30	-7	-12.5	-3.5	-15	-7	6.5	-12	-20	-8	-15
35	-5.5	-6.5	-2.5	-18	-9	0.5	0	-23	-7	-18
40	-8.5	-5.5	-3.5	-17	-12	-2.5	-26	-20	-6	-14
45	-7	-8.5	-7	-16	-8	-2	-27	-21	-6	-15
50	-8	-4	-14.5	-15	-8	-3.5	-33	-19	-4	-14
	Heart Rate min-1									
-5	-1	10	-4	-12	7	-8	-2	18	16	-3
-4	1	11	-3	-12	7	-5	-3	16	12	-2
-3	0	7	-3	-12	4	-3	-3	8	11	-1
-2	-1	5	-3	-11	0	-3	-2	5	9	-2
-1	-1	3	-1	-4	0	-2	-1	4	3	-2
0	401	359	427	421	386	440	427	452	458	401
1	-27	-25	-29	-17	-37	-19	-5	-27	-17	-5
2	-45	-30	-34	-42	-47	-23	-24	-44	-19	-14
3	-54	-32	-33	-21	-49	-26	-30	-50	-19	-15
4	-58	-33	-35	-17	-52	-27	-17	-44	-18	-13
6	-65	-36	-35	-14	-57	-34	-18	-53	-17	-8
8	-68	-39	-37	-14	-60	-41	-30	-58	-27	-15
10	-66	-40	-47	-14	-61	-44	-32	-60	-37	-18
15	-53	-40	-47	-13	-66	-55	-41	-59	-51	-26
20	-37	-47	-52	-11	-72	-27	-47	-15	-62	-37
25	-25	-53	-48	-17	-66	10	-56	-27	-47	-42
30	-15	-33	-38	-29	-48	36	-53	-52	-45	-46
35	-1	-9	-37	-32	-37	18	-11	-62	-45	-48
40	5	-15	-32	-31	-6	10	-58	-60	-42	-51
45	14	-23	-41	-25	9	6	-51	-40	-30	-51
50	25	-27	-3	-24	27	-4	-43	-30	-20	-54

ICV Clonidine (5 ug/Kg) on Heart Rate and Blood Pressure

time	Heart Rate						
min	.....			min-1	.....		
-10	2	-6	-9	-3	2	-6	0
-5	3	1	-6	-4	2	-5	0
-4	2	-5	-5	-3	0	1	2
-3	1	-2	-3	-3	0	-2	1
-2	0	1	-3	0	0	1	-1
-1	-2	0	-2	0	-2	2	-2
0	345	433	418	435	345	436	464
1	0	-7	0	0	0	-2	-1
2	-4	-24	-3	-19	-4	-14	-19
3	-6	-43	-5	-33	-9	-25	-35
4	-10	-45	-8	-45	-10	-36	-42
6	-12	-54	-10	-54	-12	-53	-58
8	-15	-62	-15	-64	-19	-66	-67
10	-20	-68	-24	-69	-20	-72	-70
15	-23	-77	-34	-80	-24	-70	-76
20	-29	-76	-49	-90	-30	-71	-87
25	-22	-63	-60	-85	-22	-71	-88
30	-17	-65	-65	-77	-18	-71	-91
35	-15	-68	-56	-78	-14	-62	-89
40	-15	-57	-47	-79	-15	-64	-85
45	-17	-47	-42	-79	-18	-62	-79
50	-13	-33	-42	-73	-13	-63	-74
55	-9	-27	-40	-71	-10	-65	-72
60	-5	-27	-40	-71	-10	-65	-72

	Blood Pressure						
	.....			mmHg	.....		
-10	0	3	2	2	-2	0	-1
-5	0.5	1	2	2	1	0	-1
-4	0.5	0	1	-1	0	3	-1
-3	-1	1	2	-1	0	2	-1
-2	-1	2	1	-1	0	0	-2
-1	-1	0	1	0	0	0	-1
0	111	103	84	81	111	83	86
1	0	-1	0	1	-1	0	2
2	-3	-2	-3	-1	-4	-1	-2
3	-7	-8	-4	-4	-5	-3	-8
4	-10	-11	-5	-6	-12	-5	-9
6	-15.5	-15	-6	-8	-20	-9	-16
8	-20	-18	-7	-11	-26	-9	-18
10	-27.5	-20	-7	-10	-26	-10	-17
15	-28.5	-23	-7	-11	-29	-13	-18
20	-30	-23	-7	-13	-30	-14	-18
25	-30	-22	-7	-7	-32	-14	-16
30	-30	-20	-5	-6	-30	-11	-16
35	-31	-17	-4	-4	-31	-11	-16
40	-28.5	-16	-2	1	-28	-9	-15
45	-35	-13	1	1	-25	-9	-12
50	-34	-12	2	2	-23	-9	-11
55	-25	-10	2	4	-27	-9	-10
60	-26.5	-12	2	2	-26	-9	-10

ICV Prepared Rat Given Clonidine IV (5 ug/Kg)

time	Blood Pressure					
min	.....mmHg.....					
-10	1	1	1	3	0	0
-5	2	2	1	2	0	0
-4	1	2	1	4	1	0
-3	0.5	2	1	4	0	-2
-2	0.5	0	1	1	0	-2
-1	0	-1	1	1	0	-1
0	124	82	83	113	86	95
1	-1.5	-20	-8	-8	-4	-14
2	-4	-25	-6	-10	-14	-16
3	-5	-17	-5	-8	-14	-17
4	-5	-18	-5	-10	-13	-17
6	-6	-29	-4	-9	-13	-18
8	-10	-29	-5	-13	-13	-18
10	-12.5	-29	-6	-11	-11	-18
15	-13.5	-24	-7	-8	-13	-16
20	-14.5	-22	-4	-10	-10	-13
25	-16	-18	-3	-9	-9	-13
30	-17.5	-26	1	-11	-8	-14
35	-20.5	-31	0	-11	-9	2
40	-17.5	-31	1	-15	-11	2
45	-17.5	-33	3	-14	-12	5
50	-17.5	-36	10	-12	-13	5
55	-17.5	-36	11	-11	-13	5

	Heart Rate				
	.....min-1.....				
-10	_	7	17	4	0	-1
-5	_	7	10	3	0	-1
-4	_	6	6	2	0	-2
-3	_	-2	4	1	3	-3
-2	_	0	1	1	0	-2
-1	_	-2	2	0	-1	-1
0	_	378	374	395	375	370
1	_	-30	-27	-18	-33	-32
2	_	-35	-31	-24	-43	-44
3	_	-33	-38	-27	-46	-52
4	_	-38	-40	-29	-49	-59
6	_	-38	-45	-32	-51	-62
8	_	-38	-46	-35	-53	-63
10	_	-36	-48	-34	-56	-63
15	_	-24	-51	-27	-58	-67
20	_	-19	-50	-27	-53	-62
25	_	-4	-46	-25	-40	-58
30	_	-6	-36	-35	-32	-55
35	_	-11	-33	-20	-32	-48
40	_	-10	-33	-5	-34	-45
45	_	-13	-33	-10	-32	-42
50	_	-12	-19	-10	-37	-40
55	_	-11	-15	-9	-37	-41

ICV Clonidine Effect on Heart Rate and Blood Pressure

time	Blood Pressure							
min	.....1.ug/Kg.....			mmHg	.....2.5.ug/Kg.....			
-5	0	-1	1	0	1.5	1	2	1.5
-4	0	0	1	0.5	0	1	1	1
-3	-0.5	0	1	0	0	1	0	0
-2	-0.5	-1	0.5	-0.5	-1	1	0	0
-1	-0.5	1.5	0	0.5	0.5	1	0	0
0	89.5	81.5	91	94	87	106	84.5	83
1	1.5	2.5	1	1.5	2	-1	0	-2.5
2	1.5	2	1	1.5	0	-1.5	-6	-1
3	1	0	0	0.5	-2	-6	-9	-4
4	0.5	-2	-1.5	-1	-4	-7.5	-10	-8.5
6	-3	-5.5	-3.5	-4	-8.5	-7	-11	-14
8	-5	-5.5	-4.5	-5	-11.5	-8.5	-14	-15.5
10	-6.5	-7	-6	-6.5	-14	-7.5	-17	-16.5
15	-9.5	-5.5	-7	-7	-18.5	-10	-19	-18.5
20	-10	-5	-7	-7.5	-20.5	-9.5	-19	-18.5
25	-10	-5	-8	-7.5	-20	-9.5	-17	-19
30	-11	-4.5	-7	-7.5	-19.5	-10	-17	-19
35	-10	-4.5	-7.5	-4	-8	-9.5	-14	-18
40	-9.5	-4.5	-7.5	-7	-16	-10	-13	-16
45	-11	-5	-6.5	-7.5	-18	-10	-13	-19

ICV Clonidine (10 ug/Kg) Effect on Heart Rate and Blood Pressure

time	Blood Pressure			
min	.....mmHg.....			
-5	0	-5	1	-1
-4	1	-1	0	0
-3	0	0	0	0
-2	0	-1	-1	-0.5
-1	0	2	0	0.5
0	129	98	114	95
1	-1	-4	-4	-3
2	-3	-5	-15	-6
3	-5	-9	-18	-11
4	-7	-16	-14	-14.5
6	-11	-24	-28	-21
8	-15	-27	-31	-24.5
10	-19	-28	-33	-27
15	-24	-32	-37	-31
20	-29	-27	-38	-31
25	-28	-22	-39	-30
30	-32	-15	-38	-28
35	-31	-11	-38	-27
40	-30	-11	-35	-25

The Autoradiographic Location Of Clonidine

Work presented in the previous chapter shows that the magnitude of the response to clonidine varies with the route of administration. This suggests that access to a site of action varies with the route of administration. Therefore comparison between the different patterns of distribution and the hypotensive effect could be helpful in locating clonidine`s site(s) of action. Areas in which a high concentration is attained with a route of administration associated with only a small reduction in blood pressure are unlikely to contain the site of action. Conversely when a large fall in blood pressure is obtained the site of action is likely to be contained within regions with high clonidine concentrations.

To pursue this approach a senstive assay for clonidine is needed. The more sensitive the assay the smaller the tissue samples required which enhances the spatial resolution. Conway and Jarrott (1980) used a radioimmunoassay capable of detecting 30 pg of clonidine which, assuming a dose of clonidine within the hypotensive range and an even distribution throughout the body, represents a few milligrams of tissue. A more common approach involves subdividing the brain into relatively large pieces (Glowinski & Iversen 1966), clearly this does not provide high spatial resolution. High resolution can be obtained if a large number of small samples are taken from known sites. Suitable techniques (Palkovits 1973) exist but their use is generally confined to removing a small number of samples. To use an onerous extraction technique in combination with a complex assay is possible but very time-consuming and therefore not practicable. This approach was rejected in favour of autoradiographic location of radiolabelled clonidine which offers higher spatial resolution than tissue sampling and although time consuming compares favourably with the alternative approach.

Autoradiography has been previously used to measure the spread of drugs after central administration (Herz et al 1970, Schubert et al 1970, Fuxe et al 1968, Grossman & Stumpf 1969).

The studies were primarily concerned with the distribution of clonidine within the CNS. The prevention of diffusion after death was considered to be a major problem especially as it was intended

to look at the time course during the onset of hypotension. The onset after IV administration is rapid. A rapid death and the simultaneous prevention of diffusion is therefore important in stopping the onset at a known time. This is most easily achieved by freezing the rat in a hexane/dry ice mixture, the alternative of removing the brain and then freezing it takes several minutes and is only applicable if diffusion is unlikely and the onset of action slow. Sectioning a whole rat requires expensive equipment which is not widely available. Access was provided by ICI through the CASE award.

Autoradiography

Autoradiography involves placing a photographic film in close apposition to tissue containing a radioisotope, the emissions produce a latent image in the film that is subsequently converted to a true image during development. The density of silver grains in the film is a function of the underlying radioactivity. Autoradiography detects the radioisotope not the remainder of the molecule and therefore fails to distinguish between the labelled substance and a metabolite containing the radioisotope. The pharmacokinetics of clonidine, mentioned in the first chapter, suggest that over the short post injection times used the majority of the radioactive marker ^{3}H will be present as clonidine and therefore can be used as a marker for clonidine.

Two radioisotopes have been incorporated into the clonidine molecule, ^{14}C and ^{3}H Both are beta particle emitters but their energy spectra differ. The beta particles from ^{14}C are of higher energy and travel further in tissue and emulsion. It follows that the distance between a silver grain and the point of origin of the Beta particle is greater for ^{14}C than ^{3}H and that the theoretical resolution is higher with the latter. Emissions from a ^{14}C source may travel 100um whilst those from ^{3}H do not exceed 3um. Therefore when a ^{14}C radioisotope is used the grain density will reflect the section thickness whilst with ^{3}H no change is apparent when the depth is increased beyond 3um. Thus with sections of 5-40um, a range used in whole body autoradiography, variations in thickness between sections is not reflected in the autoradiogram when using ^{3}H but can be a problem when ^{14}C is employed.

X ray films are generally employed when large sections are studied because the combination of large crystal size, uniformity of emulsion and ease of use outway the higher resolution obtainable with liquid emulsions. The loss of resolution is of no importance in the studies undertaken. The limited penetration of ^{3}H emissions greatly reduces the efficiency of autoradiography when X ray films are used, because they incorporate an antiscratch layer (0.5-1.0um) between the emulsion and the tissue which prevents the majority of Beta particles reaching the emulsion. As a consequence ^{14}C has been the radioisotope of choice in whole body autoradiography.

Although ^{14}C has been incorporated into the clonidine molecule it is not supplied by either New England Nuclear or Amersham International, the major radioisotope manufacturers. ^{3}H clonidine is obtainable with a high specific activity permitting the use of doses in the hypotensive range. The cost is prohibitive when X ray films are employed. Attempts have been made to increase the utility of tritium with chromatograms and macroscopic sections:

a) Applying liquid emulsions to the section (Rogers 1959, Markman 1963, Chamberlain et al 1964). This ensures intimate contact between tissue and emulsion which increases the number of Beta particles reaching the latter. Diffusion of soluble substances can occur, chemography is enhanced and the production of even layers of emulsion is difficult. Liquid X ray emulsions are not readily available.

b) Radio flourography. A scintillant generates light on exposure to Beta particles and a light sensitive film is then used to detect the latter (Wilson 1959, Farebrother & Creasy 1976). Exposure at low temperatures further enhances sensitivity (Luthi & Waser 1965) as does preexposure of the film to a controlled light flash (Laskey & Mills 1975). Problems arise with diffusion, non linearity of the response, reduced resolution and difficulties in producing low temperatures

c) Tritium sensitive X ray films. Two films have been developed CEA Verken and Sakura Marg (Makita & Hatuoka 1978), the latter is not obtainable in the UK and information scarce. The CEA Verken ^{3}H film (Larsson & Ullberg 1977a,b Applegren et al 1977) has a "monolayer" of emulsion applied to one side of the backing, uses large crystals (1.8 um), a high silver to gelatin ratio and no

antiscratch layer. This combination makes this film 12-265 times as sensitive as the commonly used X ray films when tritium is employed (Larsson & Ullberg 1977a). The absence of the antiscratch layer greatly increases the number of beta particles reaching the emulsion which is itself very sensitive to the low energy emissions of tritium.

Of the three approaches outlined for increasing the sensitivity of X ray films to tritium only the last was applicable.

Methods

Summary

1. Anaesthetized rats injected with a mixture of radioisotope and unlabelled drug. Tritiated clonidine is supplied in ethanol and 0.001 M HCl. It was redissolved in saline after blowing off the original solvent with nitrogen. Blood samples taken to determine the plasma level of radioactivity.

2. Animals killed by immersion in hexane/dry ice (solid carbon dioxide) mixture -75 °C.

3. Whole animal mounted in a block of carboxymethylcellulose (wallpaper paste) and stored frozen until sectioned.

4. The frozen block was trimmed. Mounting tape applied to the cut surface and 20-30 um sections taken with a cryostat (Bright & Sons) at -20 °C.

5. Sections were freeze dried at -20 C for 48 hrs. in cryostat cabinet.

6. Sections were removed from cryostat after placing in an air tight container containing silica gel and were allowed to reach room temperature before removing.

7. Excess tape removed and talcum powder applied to tissue and blown off.

8. In a dark room under safe light, Wratten 6B.Mounted sections were placed in apposition to film and put in a press.

9. A light tight container with film, tissue and press were left in cold store to expose.

10. Film developed in accordance with manufacturers directions, 4 mins in developer at 20°C.

The whole body autoradiography technique used is predominantly that

developed by Ullberg (1954,1977). The technique prevents diffusion of clonidine by rapidly freezing the rat and keeping sections frozen until they are dehydrated. After cutting, the sections are kept at -20^{0}C to allow water to sublime. Condensation onto the cold section by moisture in the atmosphere on removal from the cryostat is prevented by bringing the sections to room temperature in a moisture free environment. It can then be handled safely at room temperature, although care needs to be taken to prevent the section becoming damp which would reintroduce diffusion problems.

Cutting was performed on a Bright & Sons cryostat, this is essentially a powered sledge microtome mounted in a deep freeze, it is capable of cutting whole body sections of animals up to the size of small pigs. Prior to cutting the frozen animal was mounted in a block of carboxymethylcellulose which provides support. The block is trimmed until the areas of interest are revealed. Sections were cut between 20 and 30 um. As tritium was used variations in section thickness are not reflected in the autoradiogram. Prior to cutting, adhesive tape is attached to the cut surface of the block and used to lift the section off the knife face as the sledge is driven across the knife. The tape (3M 810) prevents the section breaking up and is useful in handling as contact with the tissue can be avoided.

After cutting the sections are mounted on frames and kept in the cryostat cabinet for two days. This allows removal of water by sublimation without it passing through a liquid phase which would permit diffusion of clonidine. After removal from the cryostat the tape is trimmed and talcum powder applied. A powder ladden puff is shacken above the section and the surplus removed by blowing. This is done to reduce the adhesive properties of the tape which where it is not covered by tissue will stick to the film and cause damage on separation. Talcum powder will adhere to the exposed tape surface but will be blown off other areas and does not form a layer between tissue and emulsion that would prevent Beta particles reaching the latter. A number of sections are mounted on thick paper of similar dimensions to the film. Film and mounted sections are then sandwiched between aluminium plates a slightly compressable sheet and plastic sheets and placed in a press. This when screwed down brings the film and tissue into close apposition without causing mechanical damage. Given the poor penetration of ^{3}H beta particles

this is important.

Rats of either sex in the range 150-170gm were used. Larger rats show only marginal increases in brain weight and the increasing levels of body fat and bone calcification make sectioning more awkward and require more radioactive clonidine.

Sections from each animal were used to make up one sheet for autoradiography,

Analysis

The primary method was by eye. It is easy to relate the pattern of distribution to organs and body tissues. The silver density above a tissue is not necessarily a linear function of the underlying radioactivity. A dense tissue reduces the escape of beta particles and the film/development process is saturable. However comparison of densities above the same tissue in different animals does reflect the content of tritium. Some quantification was achieved by removing tissue from the animal for liquid scintillation counting.

Experiments

1) Chemography
2) Exposure times.
3) Dose dependence of clonidine distribution.
4) Effect of a carotid arterial cannula on lateral distribution in the head.
5) IV injection.
6) IC injection.
7) ICV injection.
8) IVert injection.

1) Positive or negative chemography is the creation or removal of a latent image by the chemical action of the specimen on the emulsion. The absence of the traditional antiscratch layer in the CEA verken film exposes the emulsion directly to the tissue making chemography a possibility.

Sections from rats prepared in the usual manner but without ^{3}H clonidine were placed in contact with exposed and unexposed film.

I.V. Clonidine For Autoradiography

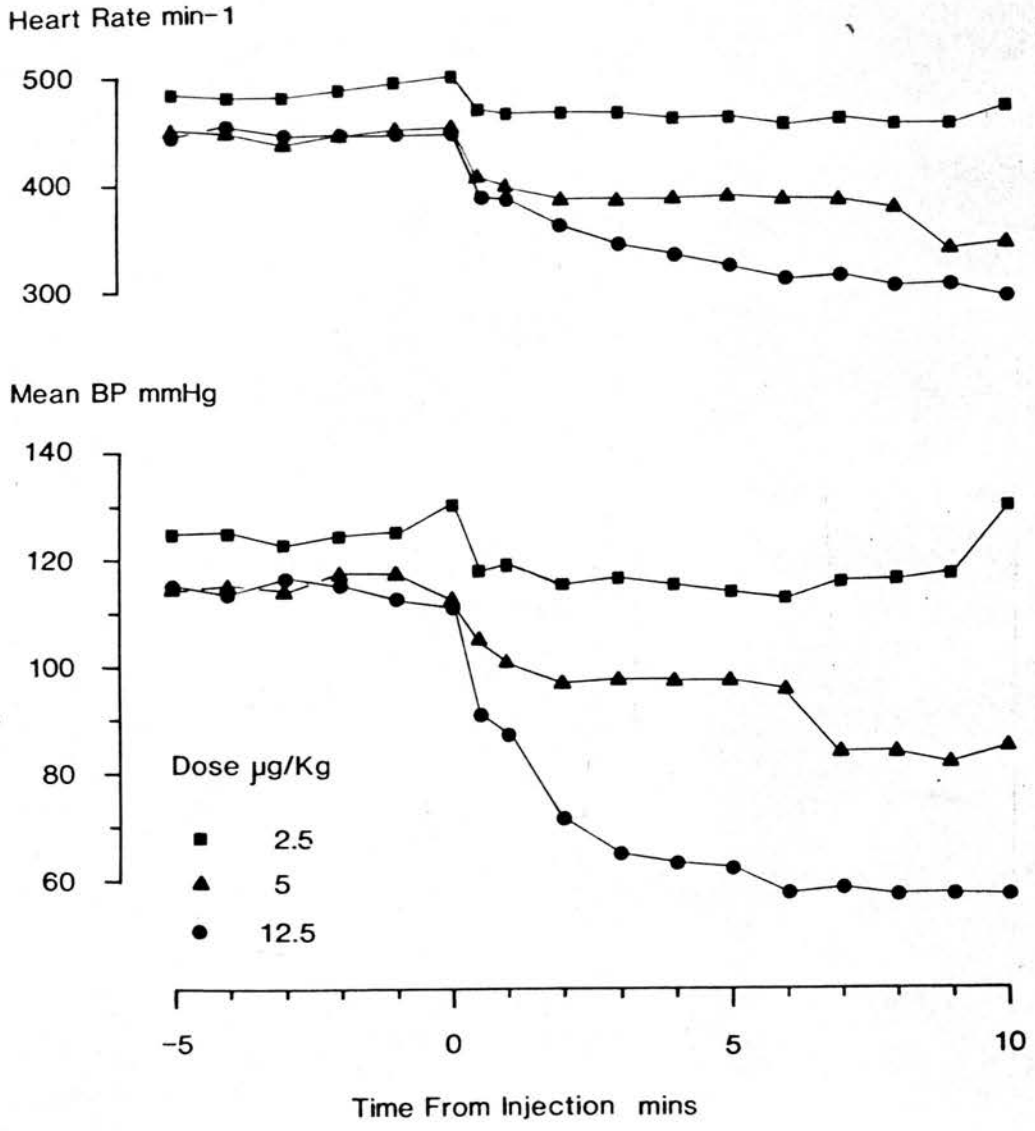


Fig. 5.1a Left Page

IV Clonidine For Autoradiography

Blood Pressure and Heart Rate Changes

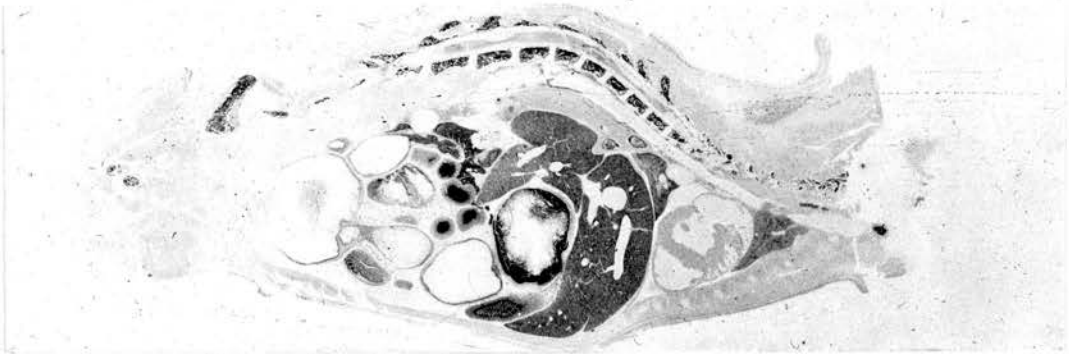
Fig. 5.1b

Distribution Of Clonidine After IV Administration.

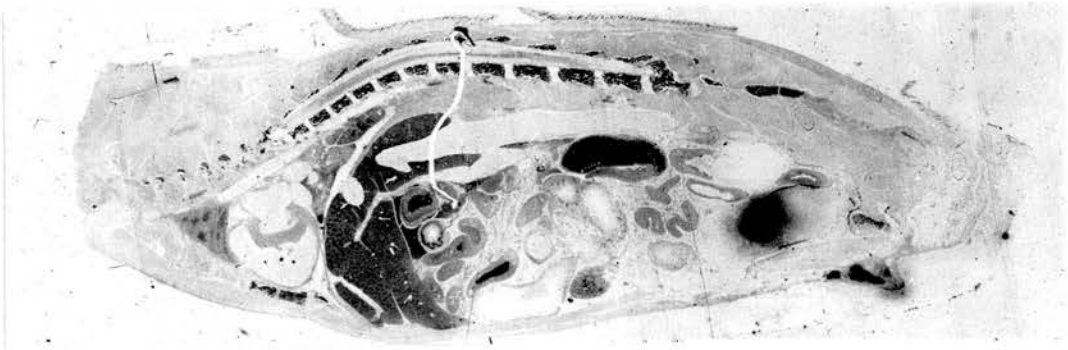
Each autoradiogram is taken from a separate animal.

Killed 10 minutes after administration.

Sections in dorso ventral plane around the midline



2.5 ug/Kg Clonidine IV



5 ug/Kg Clonidine IV

10 ug/Kg Clonidine IV

Fig. 5.1c

Autoradiogram With Labelled Structures

Animal killed at 20 minutes

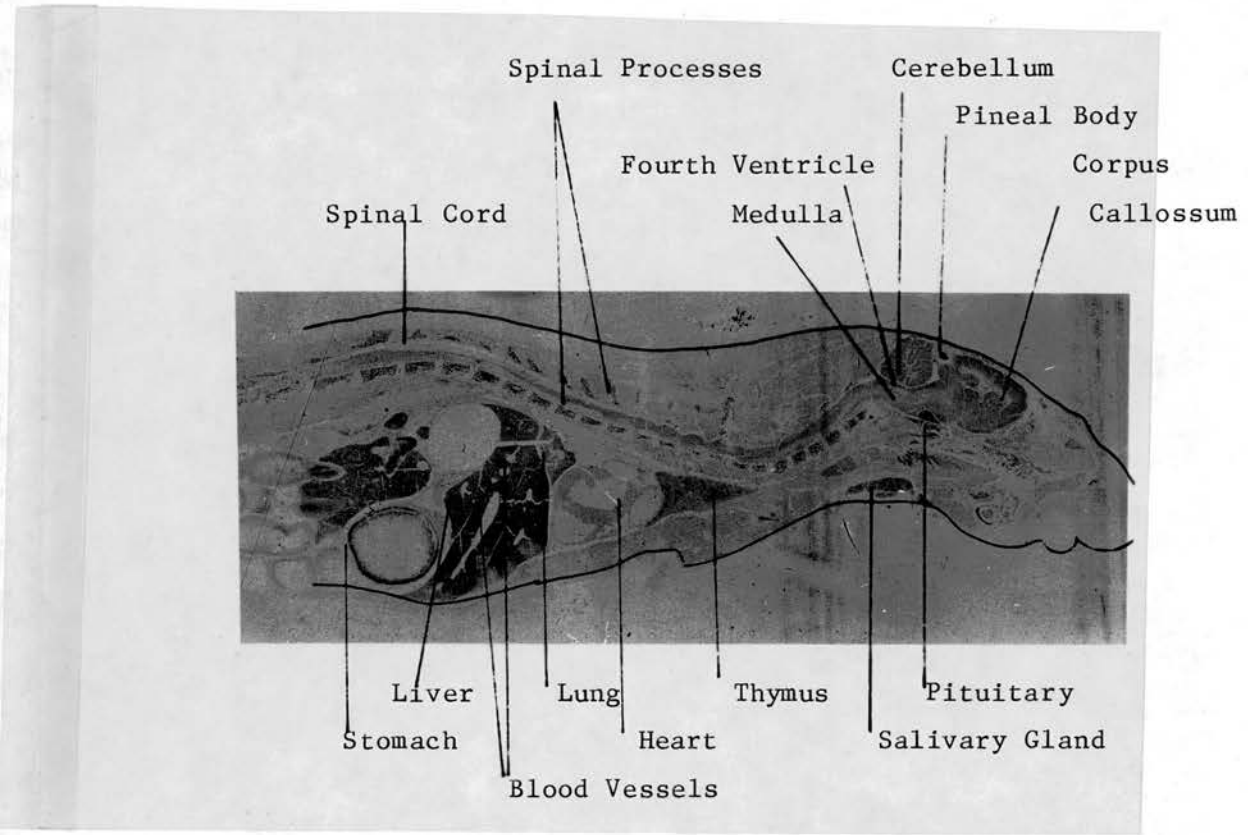


Fig. 5.1d

Longitudinal Section Away From Midline

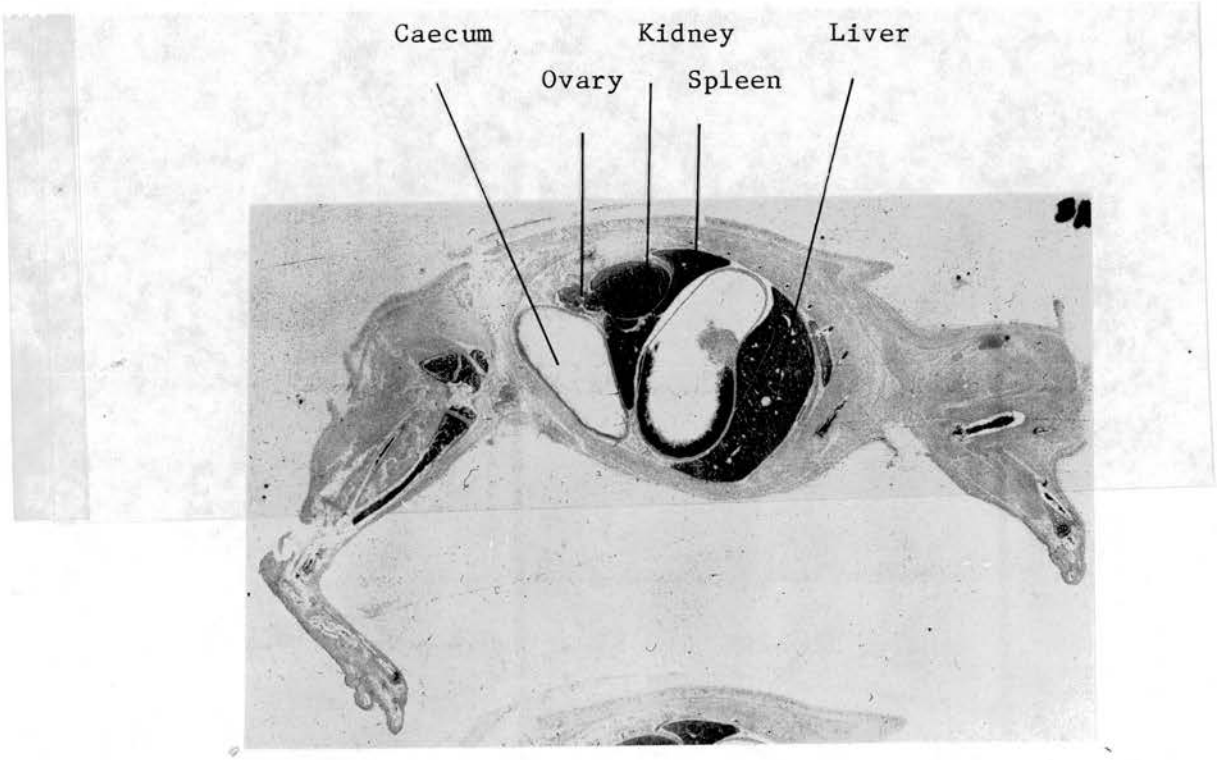
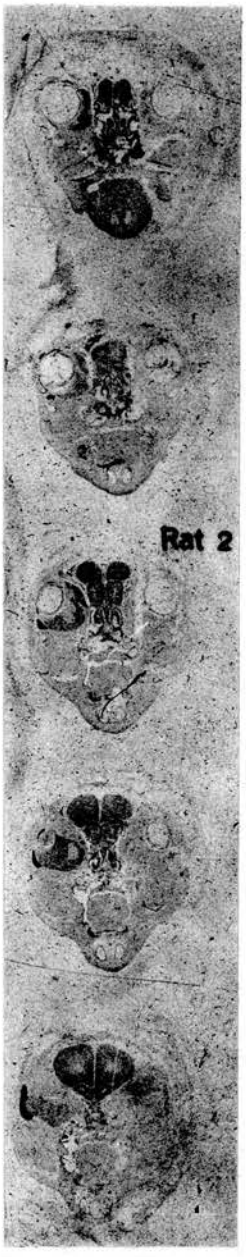


Fig. 5.1e
Transverse Section Through Head
Clonidine IV
One Carotid Cannulated



Olfactory Lobe
Tooth Pulp

Lacrimal Gland

Lacrimal Gland

2) Exposure times. The density of the developed film depends on the radioactive dose given, the size of animal used and the exposure time. Tritiated clonidine is expensive and to derive the maximum use from limited supplies only small doses were given, 40 uCi/rat. Applegren et al (1977) used 200 uCi/rat but did not mention the size of animal or exposure time. To determine the exposure time for the small doses of tritium used sections from one animal were developed after different exposure times.

3) Dose dependence of clonidine distribution. In the section on pharmacokinetics in chapter 1 it is suggested that differences in the reported T1/2s of clonidine may reflect the different doses employed. Differences could reflect a saturable transport system or be secondary to the cardiovascular changes attendant upon clonidine administration. Should they appear over the dose range giving a hypotensive response this would have to be taken into account.

The standard dose of ^{3}H clonidine was given together with unlabelled clonidine to a final dose of either 2.5, 5 or 10 ug/Kg. One rat received each dose. Dosing was via the jugular vein and blood pressure was recorded from the right carotid artery. Usually a femoral artery was used but these three animals were simultaneously used for the next experiment, the effect of carotid cannulation. The animals were killed 10 mins after IV clonidine, when the hypotensive response was fully established.

4) Effect of a carotid artery cannula on lateral distribution in the head. As mentioned in chapter 2 the pressure recorded from the central end of a ligated carotid artery is lower than that found on the cardiac side of the ligation. It is therefore possible that the blood supply to one side of the head and/or brain may be compromised by carotid cannulation. As small rats were used it is more convenient to cannulate a carotid artery than a femoral artery but is of less use if clonidine distribution is affected.

After killing the heads were removed and sectioned in the transverse plane whilst the remainder of the body was sectioned in the rostro-caudal plane.

5) IV injection. Clonidine distribution does not appear to be dose dependant between 2.5-10 ug/Kg. ^{3}H clonidine 30 uCi made up to 5 ug/Kg was given IV via the femoral vein and arterial pressure recorded from the femoral artery. Animals were killed over a range of times 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 and 40.0 mins to look for changes in distribution over a period covering the onset of the hypotensive response. Doses were rapidly given as a single bolus.

6) IC Administration. Again 30 uCi of ^{3}H clonidine was given with with cold clonidine making a total of 5 ug/Kg. The mode of delivery was that described in the previous chapter. Animals were sacrificed at 0.25, 0.5, 1.0, 2.0, 5.0 and 10.0 mins.

7) ICV Administration. 15 uCi of ^{3}H clonidine were administered in a total of 3 ug/Kg. The quantity of clonidine was reduced to account the increased hypotensive efficacy of this route and because distribution was expected to be limited. Animals were sacrificed at 1, 2, 4, 8, 16 and 32 minutes after administration of ^{3}H clonidine.

8) IVert Administration. 15 uCi of ^{3}H clonidine were given by the method described in the previous chapter. The amount used was chosen to highlight the areas reached by IVert dosing and does not reflect the hypotensive potency of this route of administration.

Animals were sacrificed at 1.0, 2.0, 4.0, 8.0 and 16.0 mins. One further rat was killed at 4.0 mins using the mode of injection described by Haywood et al (1980).

Results

Pictures taken from the autoradiograms are shown in this chapter. They were obtained using the original autoradiogram as a negative to make a contact print which was in turn contact printed. This results in a picture showing density as radioactivity in the same manner as the original. The quality has deteriorated due mostly to the uneven light source used to make the contact prints.

Rather than show every autoradiogram a few complete autoradiograms and sections from a number of others have been included. A summary table showing the results of the

autoradiographic work and the hypotensive actions of clonidine when given by different routes appears at the end of this chapter.

1) Chemography. Development of the film showed no evidence of positive or negative chemography. This is a common result when freeze drying is the only tissue processing undertaken. It follows that when ^{3}H clonidine is employed the silver grains relate to radioactivity. Mechanical damage to the film becomes apparent after development but is easy to relate the mounting tape, finger prints or scratches and can be easily separated from that due to ^{3}H.

2) Exposure times. Films were developed after 17, 35 and 70 days and the range of densities deemed satisfactory only in those exposed for the longest time. Seventy days is a long exposure time for whole body autoradiography but compares favourably with times used in autoradiography at the electron microscope level. To increase the number of experiments that could be undertaken the exposure time was extended to three months and the radioactive dose reduced to 30 uCi.

3) Dose dependence of clonidine distribution. Fig. 5.1a shows the reduction in blood pressure and heart rate in the three rats used in this section, the effect was dose dependent. Fig. 5.1b shows a section from each animal in the dorso ventral plane close to the midline. The pattern of clonidine distribution appears to be similar in all three animals. High levels of clonidine appear in the bone marrow of the spinal processes, liver and kidney.

4) Effect of carotid arterial cannula on lateral distribution. Fig. 5.1e. There appears to be no difference in the concentration achieved on the right and left side of the brain. Though in the lacrimal glands and salivary glands a higher concentration is seen on one side of the head not containing the carotid cannula. The lower levels appear on the same side as the carotid cannula.

5) IV Administration. Figs. 5.1b, 5.1c and 5.1d show sections taken in the dorso ventral plane, Fig. 5.1c is along the midline, section passes along the spinal cord, and Fig. 5.1d taken in the

same plane but at a distance from the midline. Each has an overlay used to label some of the more prominant structures.

Fig. 5.2a,b one section from each rat mounted serially on two sheets to provide an easy comparison of the changes in clonidine distribution after a bolus IV injection.

Fig. 5.2c,d A number of heads from each rat were mounted together. The letters on the facing page enable the identification of each head, 15 secs C is the section at the top of Fig. 5.2c on the binding side of the page

Brain

Less heavily labelled than the liver. Differences between grey and white tissue are very marked being lower in white areas, noticeable in the corpus callosum, cerebellum and in sections along the spinal cord. This difference persists and can be seen in sections from the animal killed after 40 mins and that killed at 15 seconds. In a number of sections the pituitary appears prominantly as does the pineal body Fig. 5.2c 1 min C. In some an area just below the the cerebellum and around the floor of the fourth ventricle seems better labelled than other areas Fig. 5.2c 1 min C.

Heart

In the sections from the animals killed shortly after clonidine injection the heart muscle appears to be the most heavily labelled tissue. Within the heart muscle a lower density is apparent, this is blood within the heart. The different heart muscle thicknesses show the right and left ventricles, Fig. 5.2a 15 seconds. During the 40 mins covered by the sections the relative density of the heart falls until it is no longer the most densely labelled tissue.

Liver/Spleen

The liver appears as a very prominant organ in the whole body sections, in respect of size and clonidine content. Areas of low density frequently appear in the tissue representing blood vessels which like blood contained in the heart contains less clonidine than he surrounding tissue. The pattern of distribution changes within the liver during the 40 mins covered by the experiment. Initially a speckled distribution is seen but this rapidly alters, by 5 minutes

Fig. 5.2a IV Clonidine Autoradiograms

Time After Administration

15 Seconds

30 Seconds

1 Minute

2 Minutes

5 Minutes

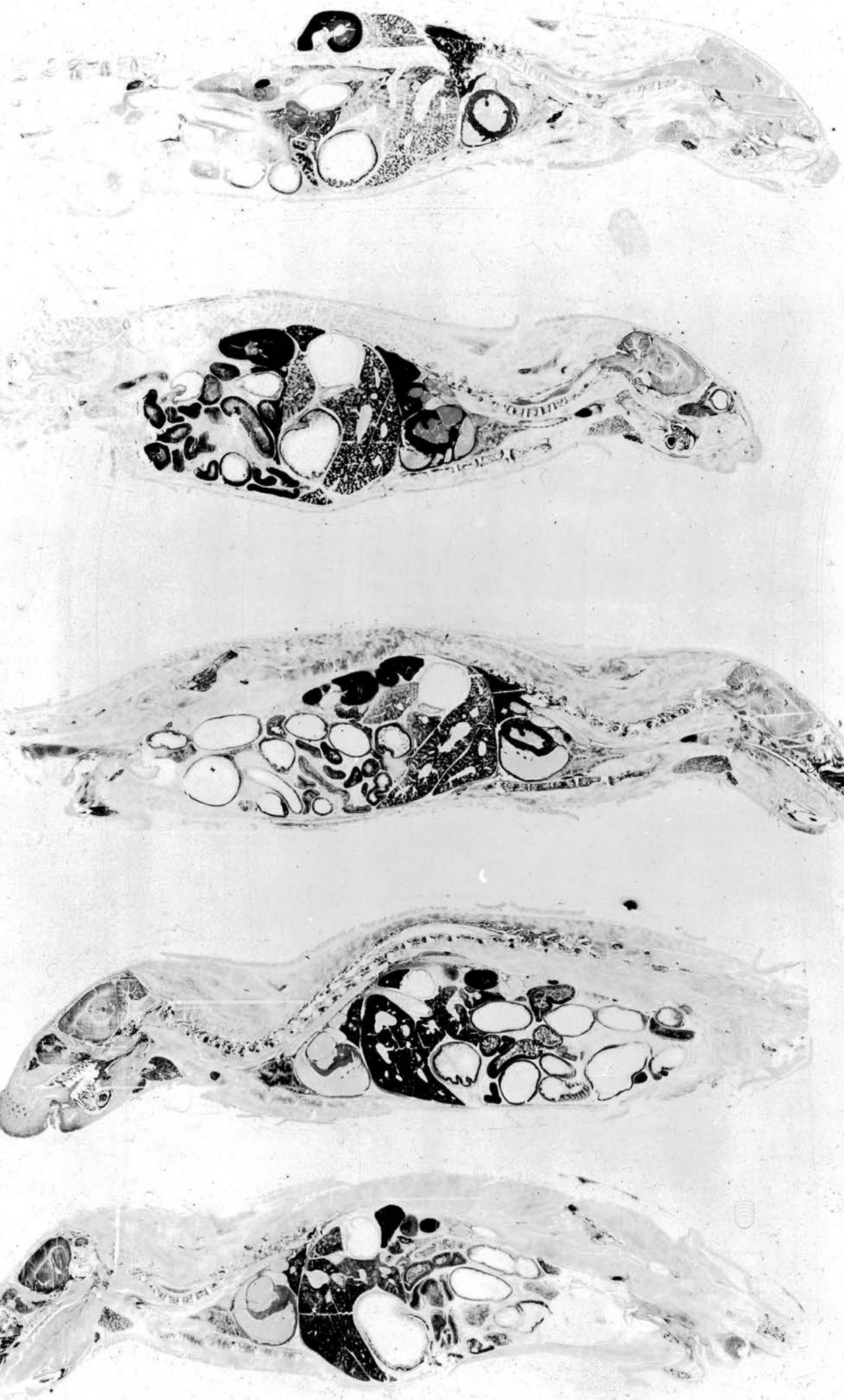


Fig. 5.2b IV Clonidine Autoradiograms

Time After Administration

10 Minutes

20 Minutes

40 Minutes

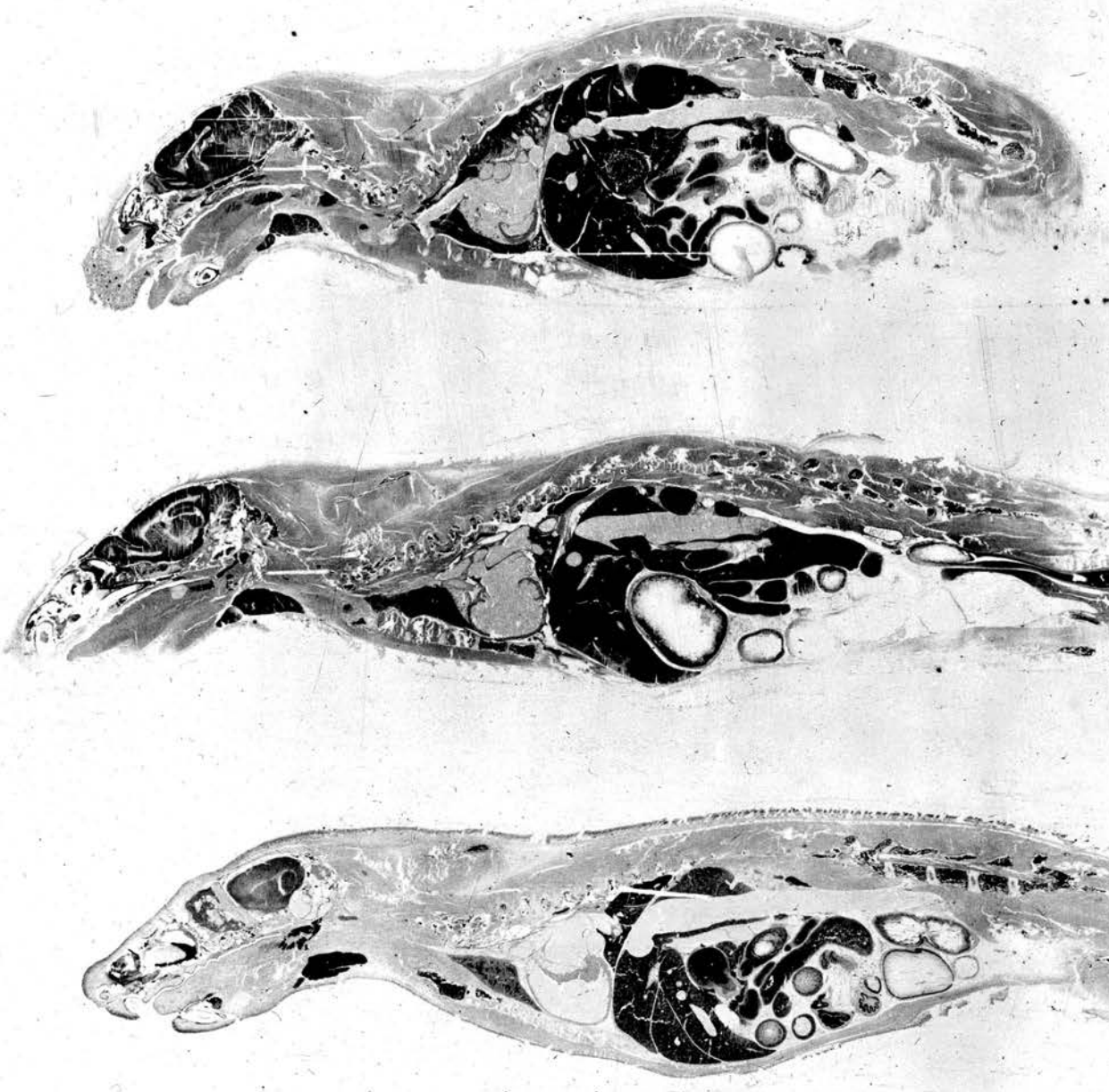


Fig. 5.2c IV Clonidine Autoradiograms Heads
Time After Administration

A B C

15 Seconds

D

A B

30 Seconds

C

A

1 Minute

B C

2 Minutes A B

A

5 Minutes

B C

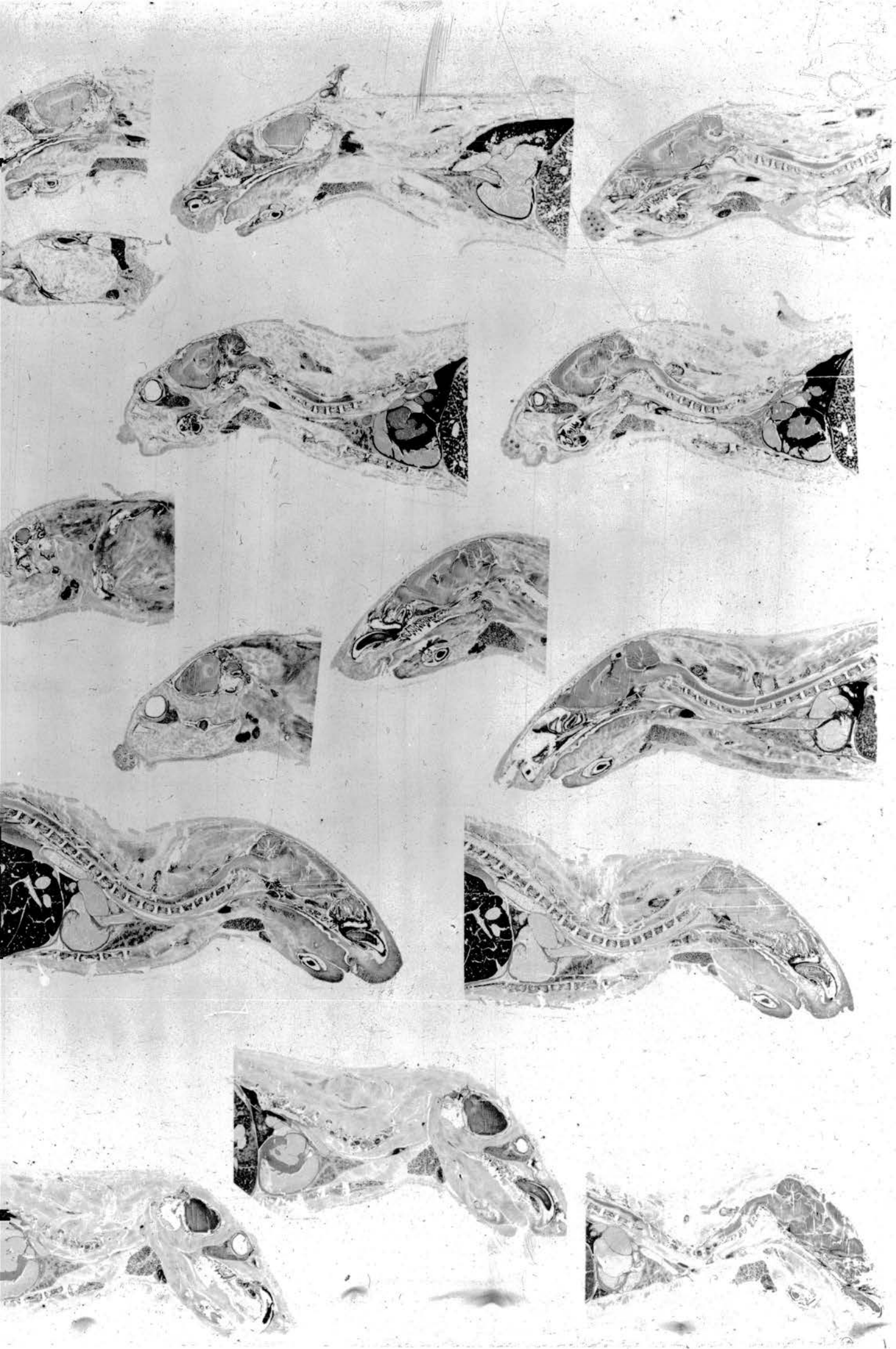


Fig. 5.2d IV Clonidine Autoradiograms Heads
Time After Administration

10 Minutes A B C

A B C

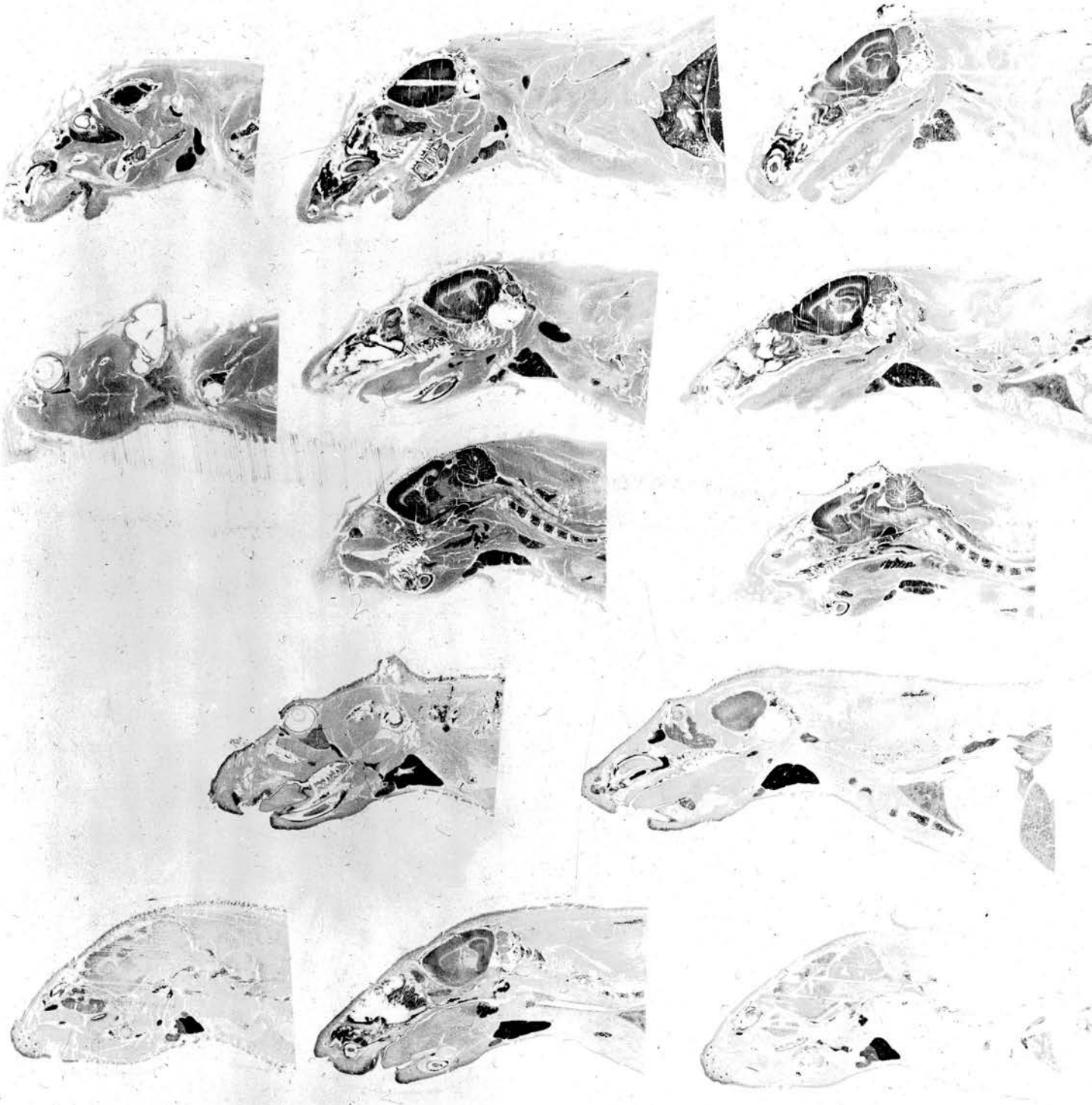
20 Minutes

D E

A B

40 Minutes

C D E



it cannot be readily discerned and later the liver exhibits a uniform density. The changes seen in the liver are similar to those in the spleen.

Lungs

The lungs appear caudal and generally dorsal to the heart and the clonidine content is initially very high but declines over the forty minutes of this study.

Stomach

The stomach is seen as a ring enclosing an area of low density, the stomach wall is well labelled especially in the region of wall thickening, Fig 5.1d. In sections from animals killed later in the experiment the labelling is starting to extend into the stomach contents and after forty minutes the stomach contents contain a high level of clonidine Fig. 5.2b 40 mins. The plane of this section does not include a large portion of the stomach, it appears below the large vein that runs parallel to the spinal cord and caudal to the liver. In sections showing more of the stomach the radioactivity does not extend into the centre of the stomach.

Small Intestine

The walls of the intestines are well labelled with clonidine but is only present in the lumen when no solid materials are present therein. Later, after 40 mins, clonidine penetrates into solid material in a similar manner to the stomach.

Feaces

Clonidine does not appear in the contents of the lower parts of the intestine.

Kidney

Clonidine very rapidly appears in the kidney in high concentrations and as in most tissues blood vessels containing relatively little clonidine appear, Fig. 5.1b bottom picture. The marked differences between the levels in the medulla and cortex abate during the period of the experiment. The whole body autoradiogram Fig. 5.2b 10 mins contains a portion of a ureter, just caudal to the kidney running

towards the rear of the animal, it is above and to the right of a black dot within the vena cava. The level of clonidine is high.

Bladder

The bladder does not appear in any of the sections shown but does contain clonidine as early as ten minutes after the IV administration of clonidine.

Fat/Muscle

Over the period of the experiment the movement of clondine into the muscle and fat progresses. In the early animals the concentration appears low and the distribution is uneven later distribution is much more uniform. In Fig. 5.5a 15 seconds an area of brown fat can be seen dorsal and slightly rostral to the heart, it is denser than the surrounding tissue.

Bone

The bone marrow attracts large quantities of clonidine, this includes the long bones of the legs, spinal processes and ribs. The tooth pulp also contains high levels of clonidine Fig. 5.2c 5 mins A. Bone and teeth are almost completely devoid of clonidine.

Salivary and Lacrimal Glands

Clonidine rapidly appears in both these tissues and maintains high concentrations until the end of the experiment.

6) IC Administration Fig. 5.4a,b,c. This pattern of distribution is clearly different from that associated with other routes of administration. Unlike the IV results the distribution is uneven favouring rostral areas of the brain, even then the distribution favours the side of the head with the cannulated internal carotid. The medulla and spinal cord are not reached in high concentrations. In areas heavily labelled the concentration is not uniform compared to that after IV administration. Parts of the head outside the brain are also heavily labelled, primarily areas well rostral. The distribution between rats differs: in the animal killed at 30 secs large areas rostral to the medulla were reached spreading into the olfactory bulb of the contralateral side, whilst

Fig. 5.3 ICV
^{3}H-Clonidine

Fig. 5.3a Left Page
Killed After 4 Minutes

Fig. 5.3b Below
Killed After 16 Minutes

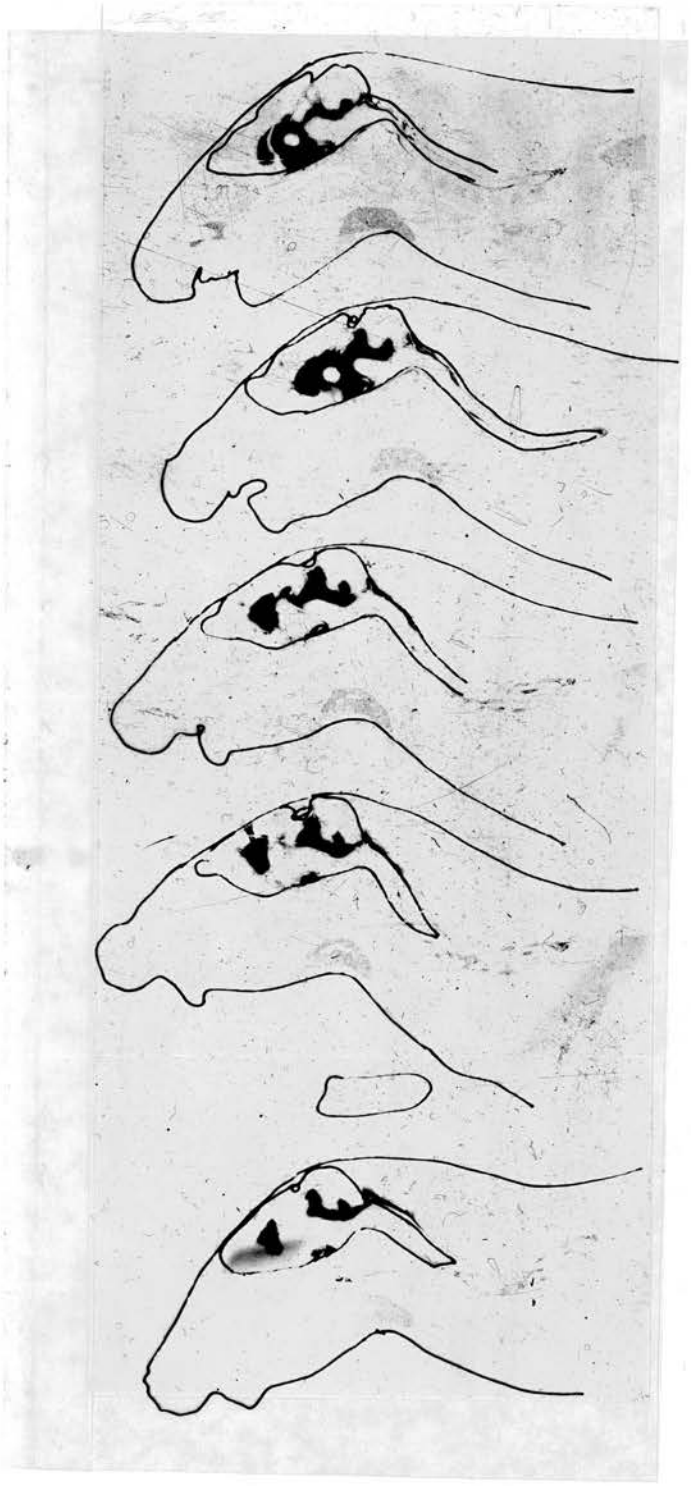
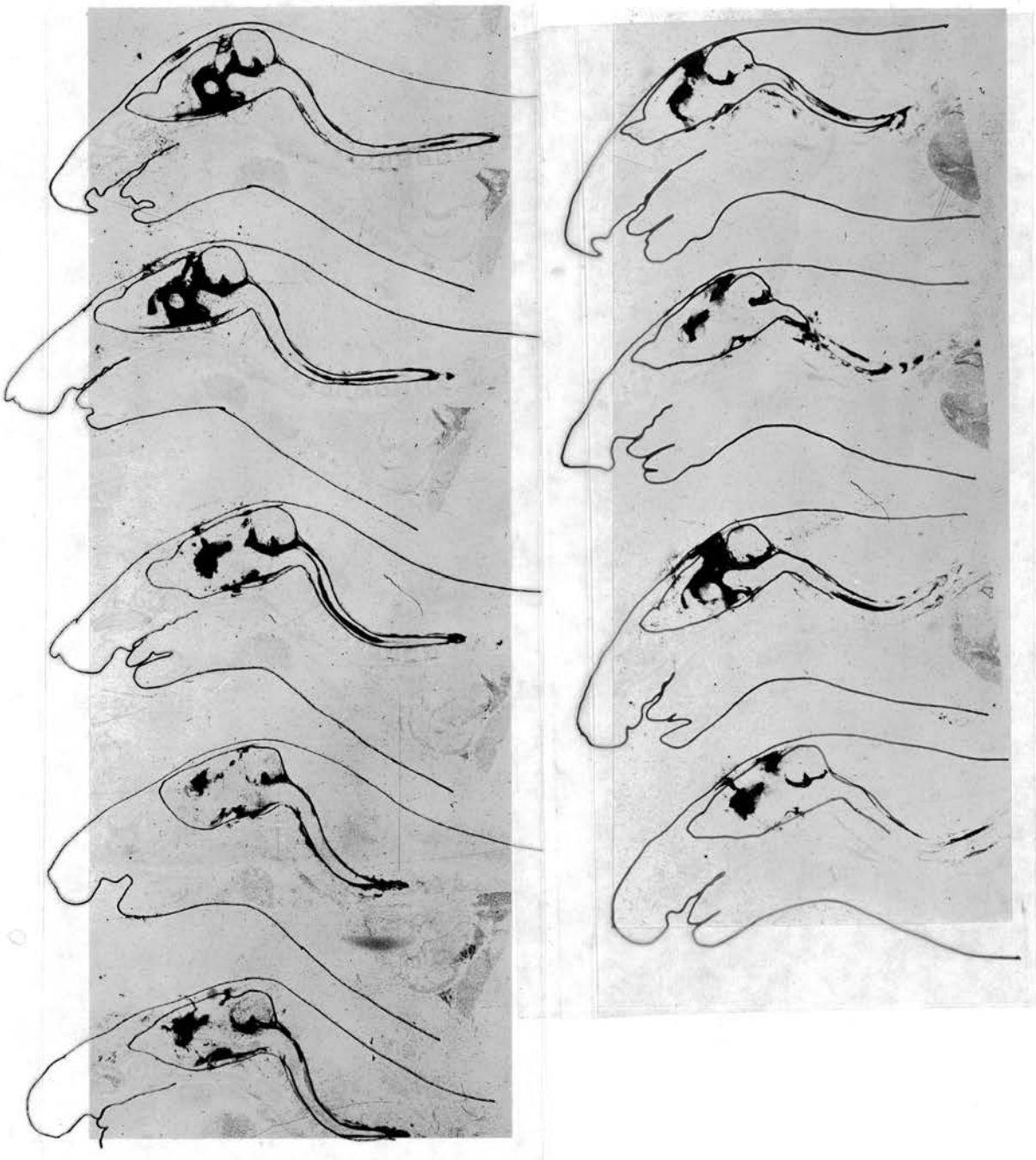


Fig. 5.3 ICV
^{3}H-Clonidine

Fig. 5.3c
Killed After 8 Minutes

Fig. 5.3d
Killed After 2 Minutes



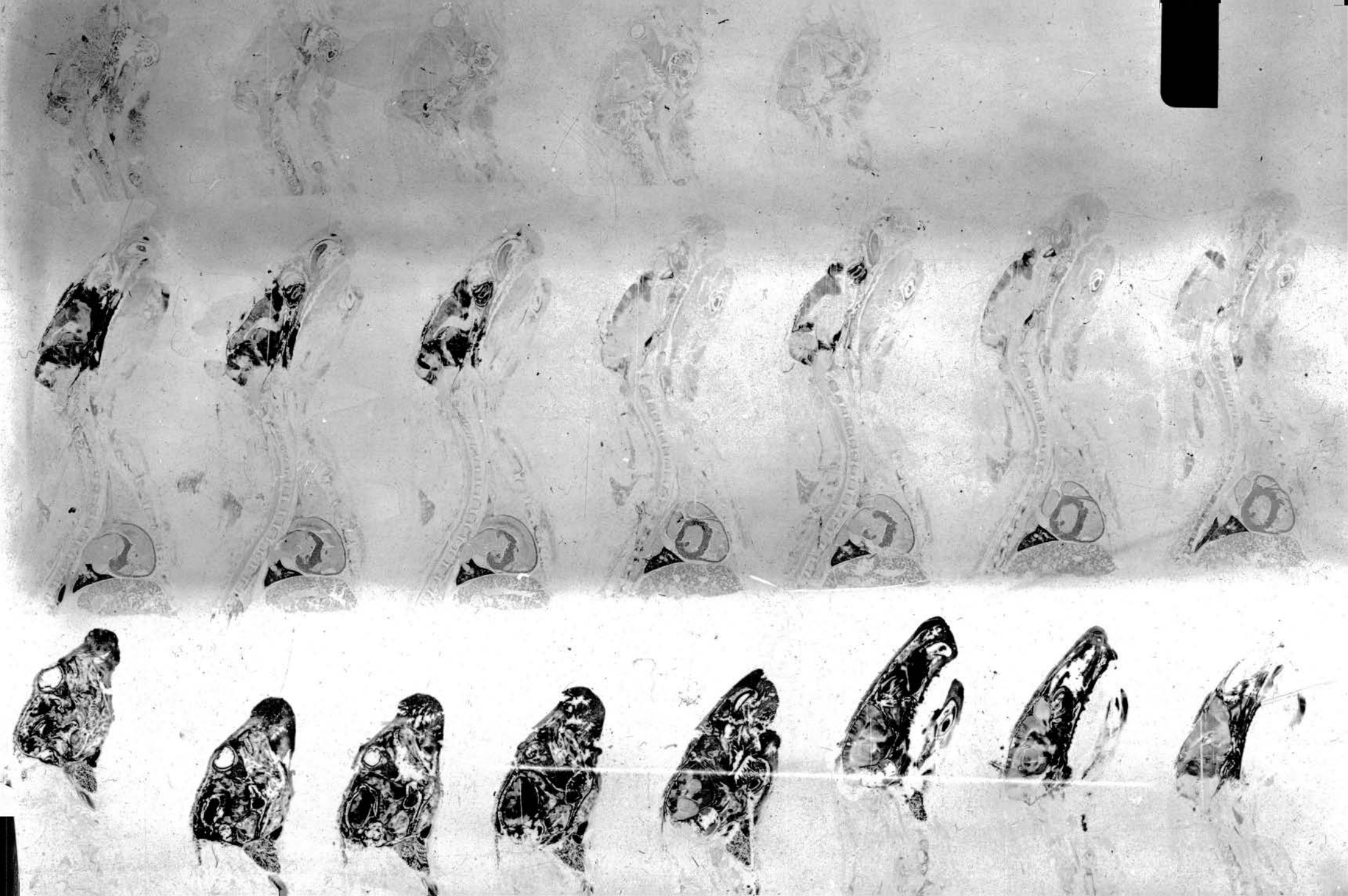


Fig. 5.4a,b,c
IC ^{3}H-Clonidine
Injection into right carotid.

Fig. 5.4a Left Page
Killed After 15 Seconds

Fig. 5.4b Over Page Left
Killed After 2 Minutes

Fig. 5.4c Over Page Right
Killed After 30 Seconds

in that killed at two minutes only a small part of the brain received clonidine in large amounts, the sections taken at 15 seconds show a greater tranverse spread of clonidine into the cerebrum than the tissue below.

7) ICV Administration Fig. 5.3a,b,c,d,. Distribution is limited to areas bordering on the ventricular system and spinal canal and the concentrations achieved in these areas exceed those associated with IV adminstration. In other areas only low levels of clonidine are seen. To facilitate visualizing the distribution an outline of the head and brain have been superimposed on the sections. The area bordering on the fourth ventricle is especially prominant. As is the mass intermedia. Penetration along the spinal cord occurs in all the animals used. Some of the sections pass along the spinal cord showing the spinal canal and diffusion outwards. The subarachnoid space is also reached with ICV administration.

8) IVert Administration Fig. 5.5a,b,c,d,e. The distribution is not uniform between animals and variable degrees of extracerebral penetration appear. Within the brain the medulla spinalis, spinal cord, medulla oblongata, pons are heavily labelled. More rostral areas have a patchy distribution and clear limits to the penetration of clonidine are seen. In section from the rat sacrificed at 16 mins the densities seen are low but higher levels are still apparent in the spinal cord and medulla. Extracerebral tissues not uniformly reached: in the 8 minute animal fig. 5.4a only a few areas bodering on the spinal cord have high concentrations of clonidine whilst those after 1 and two minutes have large areas labelled. The extracerebral areas are distinct from those seen with IC administration and are generally caudal to the medulla.

In one animal the techique of Haywood et al (1980) was used, the brain was not included in the areas labelled heavily Fig. 5.4d

Discussion

These experiments show that it is possible to locate small amounts of tritiated clonidine with the new CEA Verken film using long exposure times. No evidence of chemography was found and although the film is susceptible to mechanical damage careful

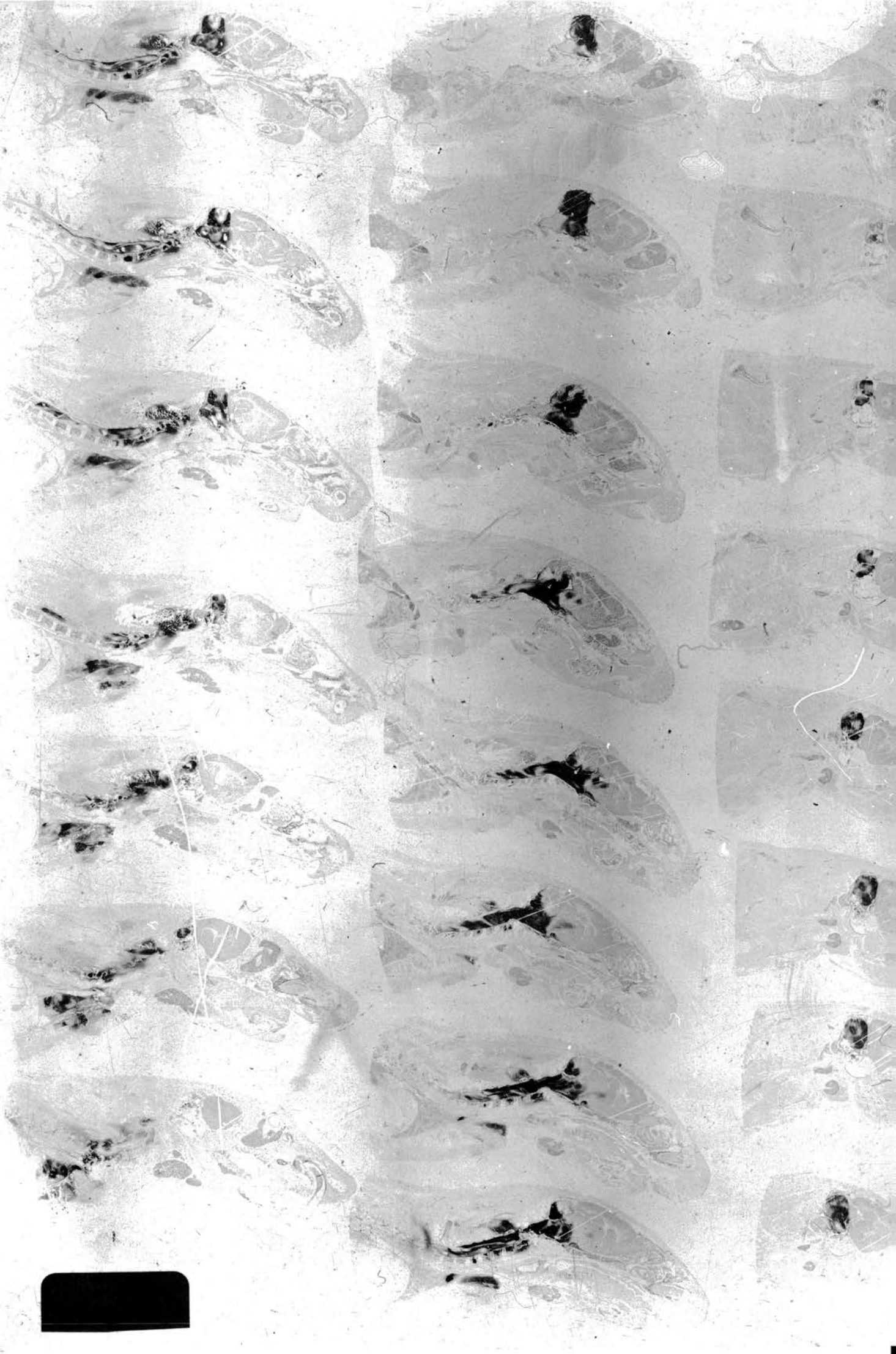


Fig. 5.5a,b,c,d,e
IVert ^{3}H-Clonidine

Fig. 5.5a Left Page
Killed After 8 Minutes

Fig. 5.5e Over Page Right
Killed 1 Minute

Fig. 5.5b
Killed After 2 Minutes

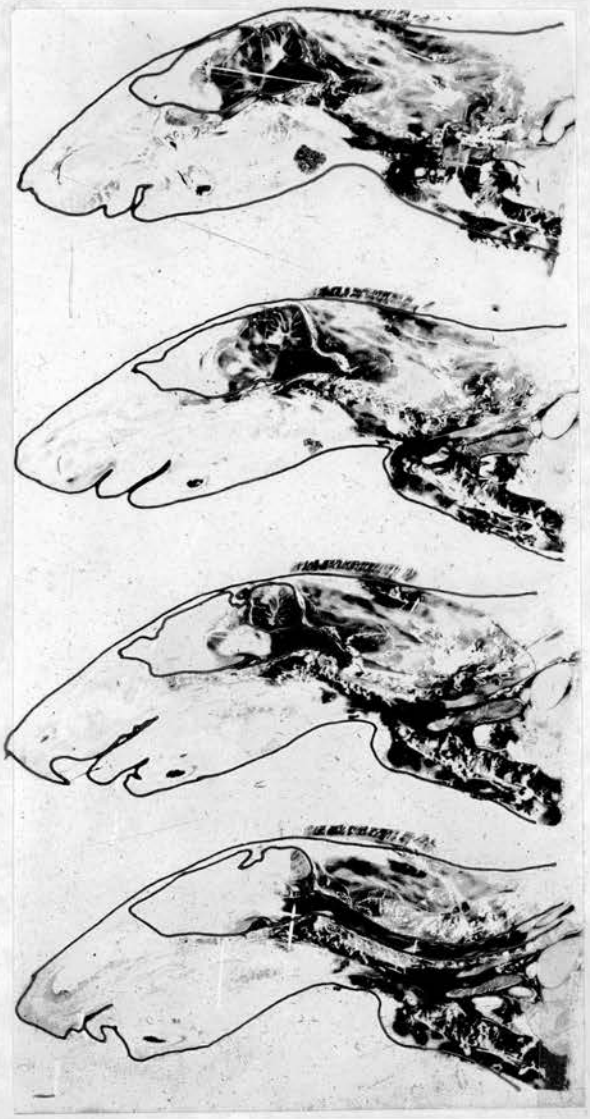
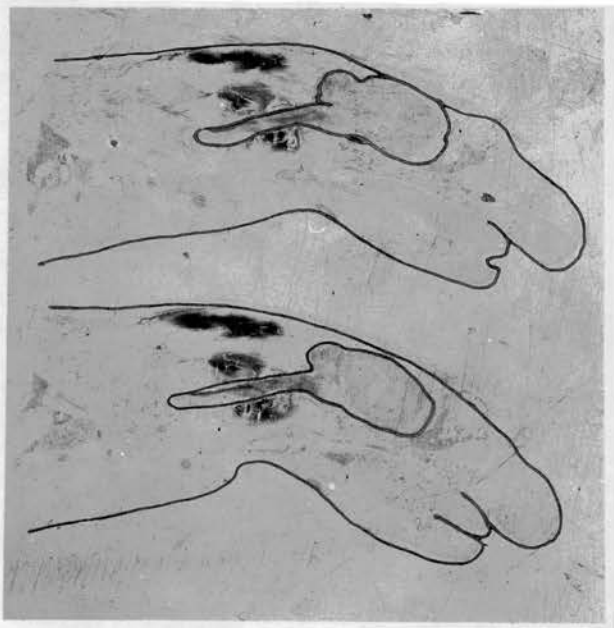


Fig. 5.5c Killed After 4 Minutes
IVert via brachial artery.

Fig. 5.5d
Killed After 16 Minutes



handling and the use of talcum powder, to reduce the adhesive properties of the backing tape, reduce damage to the film to an acceptible level. Separation of sections from the film has been done underwater, to reduce the light flashes otherwise seen when the adhesive tape is pulled away from the film. The flashes would expose the film and can be misinterpreted. Talcum powder avoids flashing and the destruction of the tissue sections seen with underwater separation.

The high specific activity (20 Ci/mmol) of tritiated clonidine permits the use of concentrations of clonidine in the hypotensive range unlike early studies on clonidine pharmacokinetics (Cho & Curry 1969).

The use of autoradiography in preference to tissue assay provides good spatial resolution but the procedure is more invovled and fewer animals can be used. In this study single animals were employed, this compares with groups of five used by Conway & Jarrott (1981) in a pharmacokinetic study on clonidine. The other advantage, better spatial resolution appart, is that all the body tissues are available for study and not just those selected for assay. Uneven distributions within tissues and the limited distribution following ICV administration would be much harder to establish with tissue assays but the quantitative data derived from assays is not readily obtained with autoradiography.

Clonidine does not reach, in equal concentrations, both sides of the head when a carotid arterial cannula is in place. This reflects the lower blood pressure seen in the cerebral end of a ligated carotid artery compared to that found on the cardiac side (see chapter 2). No transverse concentration gradient appeared in the brain suggesting that the circle of Willis compensates for the ligated artery. Despite this finding it was decided to record arterial pressures from a femoral artery and leave both carotids intact.

The three animals killed at ten minutes after administration of a range of clonidine doses, though with the same amount of radioactive clonidine, displayed very similar patterns of distribution. This means that variations in the quantity of clonidine injected are not critical and that the amount used could be adjusted for each route of administration.

The location of clonidine in the bladder and intestinal tract follows the known pattern of excretion in the rat (Rehbinder 1969, Cho & Curry 1969) in the urine and faeces. As a lipophilic drug clonidine readily passes in to the intestinal contents and similarly passes from them into the blood stream when administered orally, the usual manner. Clonidine is a basic drug and therefore its accumulation in the stomach will be enhanced. The levels seen in the stomach are the highest within the intestinal tract which support this idea. In the abscence of active transport the unionized drug concentration across a lipid membrane comes rapidly into equilibrium. The acid pH of the stomach will result in the ionization of a large proportion of the clonidine present and a higher concentration than that seen in the blood is achieved within the stomach. Penetration into the stomach contents appears to involve diffusion from the stomach wall, this suggests that gastric motility is low, possibly an anaesthetic effect.

The concentration of clonidine in the blood is lower than that seen in most tissues, even in the animals killed early in the experiment.

In the spleen and liver the pattern of distribution changes during the experiment. Initially it is patchy but this is lost in the subsequent 5 minutes. A consequence is that calculation of the T1/2 of clonidine in the liver over this period is misleading as it refers to the changes throughout the tissue taking no account of alterations in the pattern of distribution. Similarly the pattern of distribution alters in fat and muscle.

The initial distribution of clonidine seems to reflect tissue blood flow, shortly after administration the kidney, liver, heart and lungs are seen to contain high concentrations of clonidine. This decreases with time whilst in less well perfused tissues, muscle and fat, the concentration seems to increase over this period. Comparison of the tissue concentrations between 2 and 10 minutes reported by Conway and Jarrott (1980) supports this interpretation, although not mentioned by the authors. In the lung and heart only about 60% of the quantity of clonidine present at 2 mins was retained after 10 minutes, in contrast muscle and adipose tissue and muscle retained about 80%.

IV administration achieved high concentrations in some areas,

pineal body, pituitary and possible parts of the floor of the fourth ventricle. These areas are considered to have a less well established blood brain barrier and the resulting higher concentrations indicate that the blood brain barrier does restrict the access of clonidine to the remainder of the brain. The differences between the grey and white matter in the brain and spinal cord probably reflects the lower blood flow of the latter. Even in the animal killed 15 seconds after the injection of clonidine the drug is well established in the brain and the onset of central actions is not delayed by slow penetration into central sites. Interestingly the concentration in the brain does not decline over the first few minutes. Svensson et al (1975) found the central actions of a small IV injection of clonidine decreased over a few minutes. In the introduction it was suggested that this could represent the egress of clonidine from the brain over this time period. The lipophilicity of clonidine ensures its rapid penetration into the CNS and as the initial distribution of clonidine appears to reflect tissue blood flow these two factors would lead to high central concentrations. The pattern of distribution subsequently alters ceasing to reflect tissue blood flow and net movement from the brain might be anticipated with a reduction in central actions. This would appear as a recovery in blood pressure over the first few minutes after injection, a pattern of response not encountered.

The pattern of distribution, within and outside, the brain after IC adminstration varied between animals. However the method of administration used achieved the desired objective, reaching only a circumscribed area of the brain. Marked differences appear in the concentration achieved on each side of the brain which indicates that each carotid preferentially supplies its own side of the brain. When one carotid is occluded both sides of the brain are supplied equally. This could provide a method of delivering drugs to rostral brain areas without complications with tranverse concentration gradients. Areas outside the brain would have been reached through branches of the internal carotid.

ICV clonidine only reaches very limited areas of the brain on the edge of the ventricular system. This finding was also found by Chalmers & Wurtman (1971) using ^{3}H noradrenaline, Schubert et al (1971) with ^{3}H fentanyl and differential depletion of central

catecholamines was reported after ICV 6OHDA administration (Chalmers & Reid 1972).

IVert administration of clonidine reaches areas in the caudal portion of the brain with a preference for the side whose artery was cannulated. This does not support the findings of Wellens et al (1976) where the vertebrals were deemed to be of no importance in the rat. Differences in the size of animal used may reconcile the two sets of results. The different method used to inject intravertebrally in the rat does work and is much easier to use than the technique used in the cat and dog. However other arteries are involved and it is therefore impossible to say whether it is the vertebral that supplies areas surrounding the spinal cord. The pattern of distribution is similar to that reported for the dog and cat (Wellens et al 1975, Reneman et al 1974). The greater concentration found on the side of the cannulated carotid has been reported (Sherbin-Schepers 1979) Porsius (1980) Porsius & Van Zwieten (1978). The technique has been improved by ligating the other vertebral artery (Sherbin-Schepers 1989) and infusing drugs into both (Porsius 1980), neither has been attempted in the rat and only ligation seems feasible. In the rat the vertebrals supply the upper part of the spinal cord, it is unlikely that that this is invovled in the cardiovascular response since sympathetic preganglionic cell bodies are not found in this area. The animal killed sixteen minutes after IVert adminstration of clonidine showed only slightly raised amounts of clonidine in the medulla and spinal cord. This may represent escape of clonidine from the brain after its first pass uptake but seems to have occured much faster than the recovery in blood pressure reported with this route of administration (see previous chapter). This is a general problem with single animals and compounded by the wide variations in distribution seen between animals. The lability of the rat cardiovascular system and circle of Willis in particular mentioned by Greene (1935) may in part explain the differences between animals.

The different patterns of distribution were correlated with the differing hypotensive and heart rate-reducing effects, see previous chapter.

ICV was found to be slightly more potent than IV but in the

areas reached the concentration appears to greatly exceed that seen with IV administration and if they include the site of hypotensive action a much greater fall in blood pressure would be anticipated. It seems that IC administration does not simply provide another route of access to a central site of action but lowers blood pressure through a different mechanism, see discussion in chapter 1. ICV administration either fails to reach areas of the brain or achieves a high concentration and in no area approximates to the concentrations seen with IV administration. Actions on heart rate and arterial pressure are similar but cannot stem from an action at a common site. The periventricular areas reached by ICV dosing will include those reached following topical clonidine on either the floor of the fourth ventricle and the ventral surface of the brainstem. The high levels but relatively poor hypotensive effect suggests that these areas are unlikely to be involved in the hypotensive response to IV clonidine and that they represent a different and unrelated mechanism.

IC administration is no more potent than IV in lowering arterial blood pressure and heart rate. It follows that areas that achieve a level of clonidine in excess of that seen with a equipotent IV dose are not candidates for the hypotensive site of action. This appears to preclude the forebrain supporting the conclusions drawn from decerebration experiments.

IVert clonidine concentrates in medullary areas and lowers blood pressure potently. Higher levels of clonidine are seen in thse areas than with IV dosing and reflect the size of the IVert dose. When this is considered it follows that the site of action is contained within the areas reached after IVert administration.

There is overlap with areas reached after ICV and IVert administration which can be excluded as potential sites. Similarly areas reached by both IC and IVert routes are not candidates.

On the basis of differential distribution and hypotensive potency the central site of clonidine's hypotensive and cardioinhibitory action lies within the medulla but not on the edges of the ventricles. This conclusion is incompatible with the floor of the fourth ventricle and area S as sites of action. It is not suggested that locally applied clonidine, in these two areas, does not lower blood pressure but that this action is unrelated to the

effects of IV administration. This in turn is seen as representing the actions following oral administration. The area S has not been established in rodents but if a homologous structure exists these studies argue against its importance.

Summary Table Of Data From Chapter 4 And 5

	Route of Administration of Clonidine			
	IV	IC	ICV	IVert
Hypotensive Potency*	1	1	1.5	20
Distribution of Tritium&				
Pons/Medulla	2	2	0	8
Medulla Spinalis	2	2	0	8
Rostral to Pons	2	7	0	2
Periventricular Areas	2	2	10	2

* Hypotensive potency compares the ablility of clonidine given by each route of administration to lower blood pressure and heart rate with that following IV injection. Numbers greater than 1 indicate greater potency.

& This is measured semi quantitatively with 0 reflecting a negligible density and 10 a very high density.

Plasma Concentrations Of Clonidine

The plasma concentration of clonidine is reported to fall rapidly after bolus administration (Conway & Jarrott 1980) (Jarrott & Spector 1978). Reports of the T1/2 of clonidine vary widely, from tens of minutes to hours (Chapter 1 Pharmacokinetics). Comparison with the cardiovascular effects would be interesting. In addition measurement of the escape of clonidine from the ventricular system into the circulation would show if this could explain the response to this method of administration. Applied ICV clonidine exhibits a slow onset of hypotension and bradycardia. The peak levels slightly exceed those following IV administration and with injections of 5 ug/Kg the ratio is 1.5:1. It is possible that the slow response partly involves escape of clonidine from the ventricular system into the general circulation. This would permit peripheral hypotensive actions and access to areas of the brain not attainable from the ventricular system which would appear in addition to any central actions generated from periventricular areas.

Methods

Collection Of Blood

Arterial blood samples were taken from a femoral arterial cannula. Short cannulae were employed and sufficient blood to compensate for dead space allowed to escape before a sample was collected. Blood escaped under arterial pressure. 500 units of heparin were given per animal to prevent blood coagulation.

Treatment Of Samples

Blood was centrifuged to separate cells from plasma. 10 ul plasma samples were made up to 1 ml with water added to 10 ml of Fisofluor "2" and counted for tritium using a liquid scintillation counter. Background subtractions were made automatically from blanks prepared with distilled water instead of plasma. Conversion from counts per minute to disintegrations per minute was also made automatically. Counting efficiency was measured with an external standard.

1) IV Clonidine.

Blood from 5 rats given 5 uCi ^{3}H clondine made up to 5 ug/Kg

was collected at 0.25,0.5.1,2,5,10,20,40,60 mins. Blood volumes of 30 ul were taken at each time of collection.

2) ICV Clonidine.

Blood was taken from three rats used in the autoradiographic study reported in the previous chapter. They were killed at 8,16 and 32 mins. Each animal had received 15 uCi of ^{3}H clonidine. Blood was taken at 0.5,1,2,4,8,12,16,20,25 and 30 mins from the rat killed at 32 mins, up to and including 16 mins for the rat killed at that earlier time and up to 8 mins from the animal killed at 8 mins.

3) IVert Administration

Blood from one animal used in the autoradiograhy experiments was collected.

4) Plasma concentrations of clonidine were calculated from the amount of radioactive clonidine and unlabelled clonidine injected, the size of the animal and the plasma levels of tritium.

Results

Liquid Scintillation Counting

Background counts were 15.5 DPM, standard error 2.8 (N=6). Counting efficiency 30-33%.

Blood Samples

No problems were encountered in obtaining arterial blood samples. No alterations in the blood pressure record accompanied sample collection. Over the collection period the total blood lost by each animal would not have exceeded 0.5 mls, about 6% of the total blood volume.

1) IV Clonidine Fig. 6.1,2 and Table 6.1

Fig 6.1 shows changes in mean arterial pressure and plasma tritium concentrations following IV bolus clonidine. Plasma levels fell precipitously over the first two minutes and stabilized at about 25% of the peak level,found at 0.25 mins. Thereafter decline is slow hardly altering between 10 and 60 mins. The standard errors fell over the first four plasma samples.

Mean arterial blood pressure follows the usual pattern, briefly rising then falling rapidly to a maintained level. A slow recovery appears after twenty minutes.

Once blood pressure and the plasma tritium concentration have stabalized at 10 minutes the plasma concentration of clonidine is calculated to be 4 ng/ml.

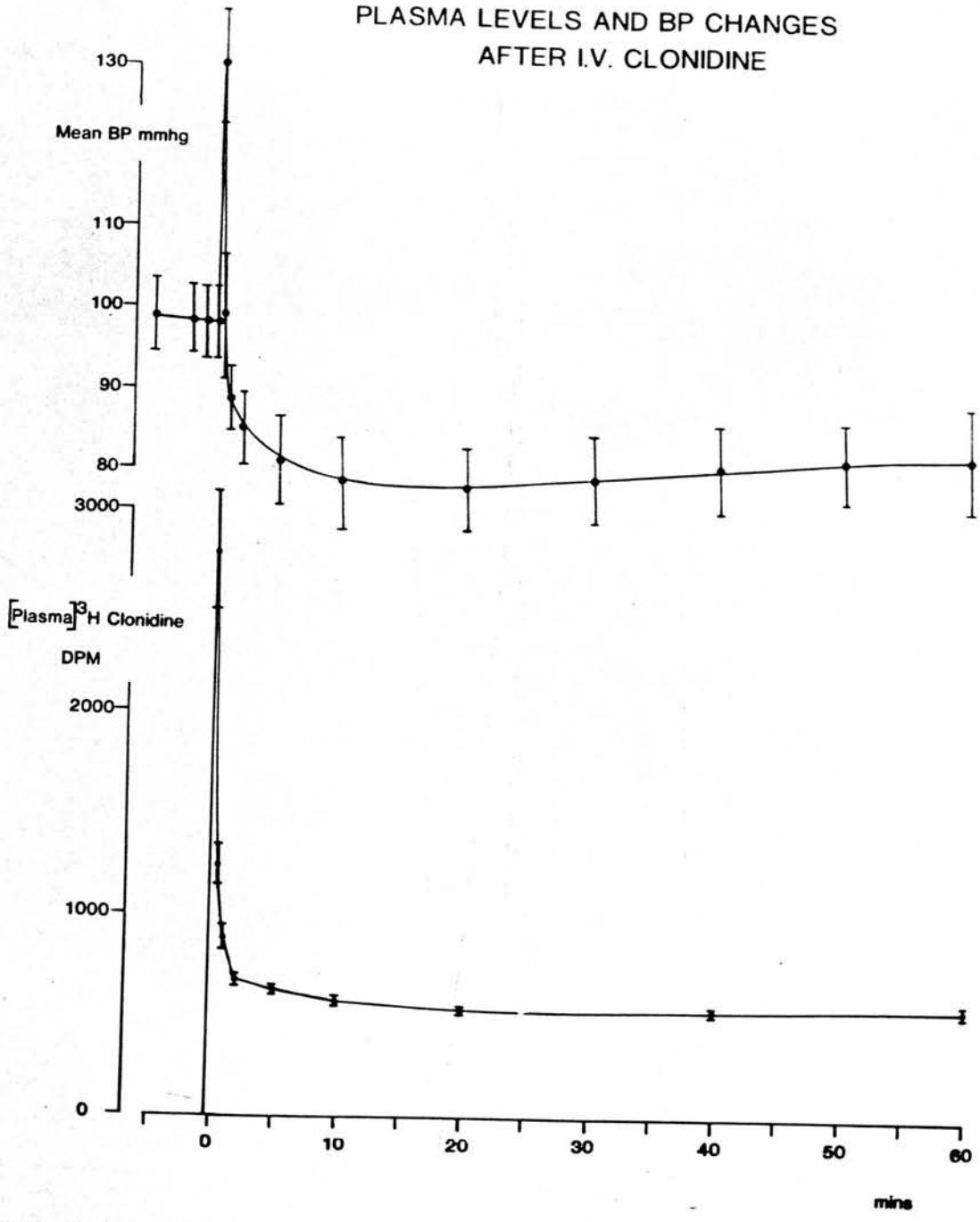


Fig. 6.1

Plasma Levels And Blood Pressure Changes After IV Clonidine

5 ug/Kg IV Clonidine with 6 uCi.

N=5

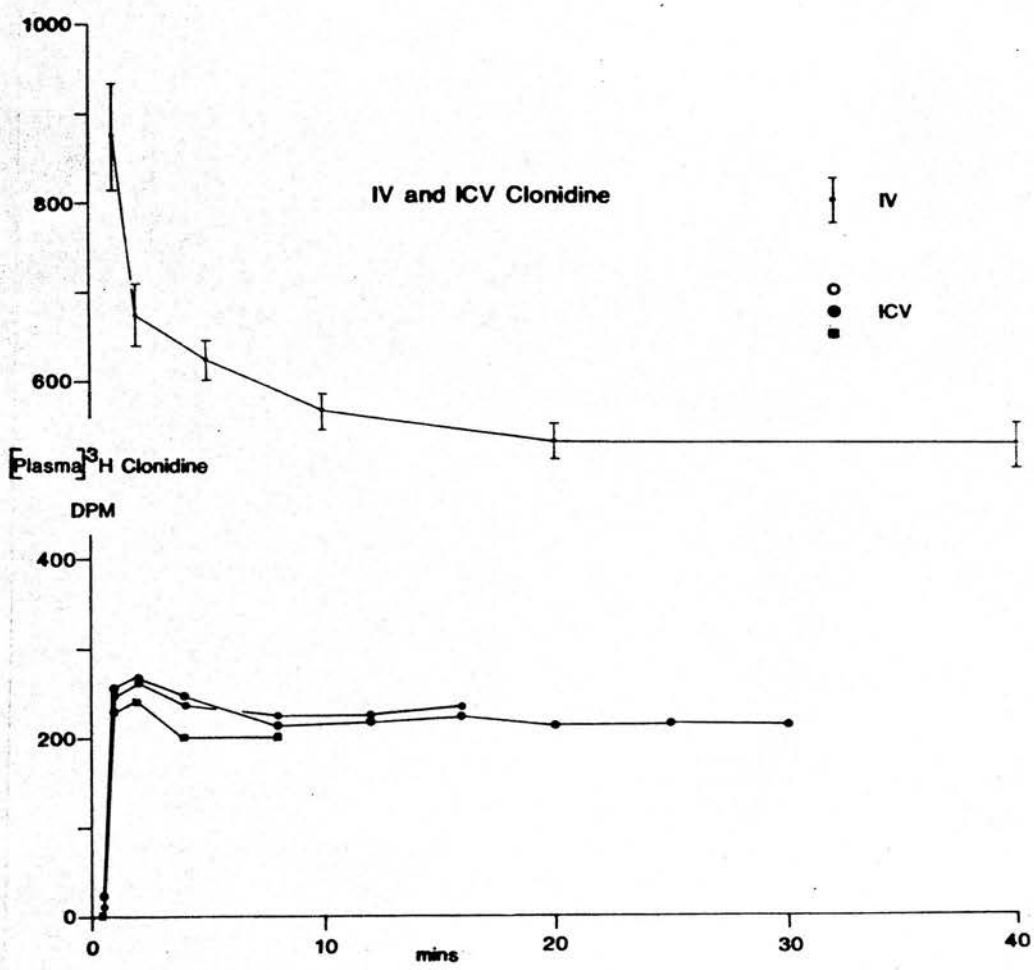


Fig. 6.2

Plasma Levels Of Clonidine After IV And ICV Administration

N=5 for IV clonidine

3 rats given ICV clonidine

2) ICV Clonidine Fig. 6.2 and Table 6.1

Fig 6.2 shows that the plasma concentrations following ICV administration of clonidine are much below those after IV. The pattern of change is also different, there is no initial peak and the concentration is stable after the first sample.

1) IVert Clonidine Table 6.2

The plasma concentration was initially high and fell very slowly, a pattern unlike that seen with either ICV or IV administration. The autoradiograms show that the tritium concentration in the medulla and spinal cord were only slightly above those found in rats after IV ^{3}H clonidine administration.

Discussion

The brief hypertensive episode following bolus IV administration coincides with the peak plasma concentration, both fall rapidly and at 30 seconds the concentration of tritium in plasma is less than half that seen at 15 seconds and blood pressure has fallen to the preteatment level. This close temporal relationship suggests that the two are related though it does not indicate whether a central or peripheral action is involved. The standard errors of the plasma tritium concentrations fall over the first few samples. This probably reflects the rapidly altering concentration which makes the exact timing of samples critical. As a percentage of the mean concentration the standard error is 10% at 0.25 seconds and 4% between 20 and sixty minutes.

The rapid drop in plasma concentration following IV bolus injection suggests that the secondary hypertension, reported in chapter 2, is unlikely to be a direct effect of clonidine on the vasculature. It may involve catecholamine release and an interaction with the adrenal gland had been reported in the urethane anaesthetized and unanaesthetized rat (Zandberg 1977) though the interaction with the barbiturate anaesthetized rat was reported to be much smaller in magnitude.

The stable plasma concentrations of clonidine found between twenty and forty minutes were surprising high, 4 ng/ml, and a reduction over this period was anticipated. Conway & Jarrott found T1/2s for most tissues in the range 40-110 mins yet in this study the plasma T1/2, not calculated, would be very long. The two studies

are similar in that the same anaesthetic, inactin, was employed. Conway & Jarrott used a smaller dose of inactin, 100 mg/Kg IP, a larger dose of clonidine 20 ug/Kg and found the return towards the pretreatment arterial pressure to be more rapid than seen in this study. This may explain the discrepancy. An alternative explanation is that metabolites of clonidine are appearing in the plasma. Tritium was used as a marker for clonidine and a metabolite containing tritium would not be detected separatley, inflating the clonidine concentration. Jarrott & Spector detected 4 hydroxy clonidine in plasma after 60 minutes, it is the main metabolite of clonidine in the rat. Depending upon the mechanism of hydroxylation the tritium might be retained in the metabolite. The metabolite is not lipophilic and therefore concentrates in blood rather than in tissues and is may have inflated the later plasma clonidine figures. This is unlikely to be a problem over the early part of the experiment as the metabolite was only reported after 60 minutes. In three of the five rats used in the IV plasma concentration study a final blood sample was taken at 80 minutes. In each case the concentration of tritium was higher than that at 60 minutes, the increase was by about 13% of the 60 minute concentration. This suggest that the tritium lablel is retained in a metabolite. This falls outside the period used in the autoradiographic studies and does not bring their validity into question.

Since the plasma concentration of tritium does not continue to fall precipitously after the first 2 minutes the use of cumulative injections of clonidine is seen to be reasonable.

After ICV clonidine only low concentrations of clonidine appear in the plasma. The much lower levels seen at 0.5 min reflect the slow injection of clonidine into the lateral ventricles but the low steady level probably represents very limited escape from the ventricular system. The plasma concentrations are about 1/3 of those seen after IV administration, once the level has stabilized.

This precludes important peripheral actions for clonidine given ICV and indicates that most of the administered drug is confined to the ventricular system. These results confirm those from the autoradiographical studies. The combination of limited escape and distribution predominantly in periventricular areas suggests that the concentration of clonidine in these tissues is very much higher

than that seen with IV administration. Given IV only 2% of the total administered resides in the brain (Timmermans et all 1977). Assuming that the plasma concentrations represent an equilibrium between tissues and blood and that the clonidine that has escaped from the ventricular system is also at equilibrium but at 1/3 the IV level. It follows that around two thirds of the administered clonidine is still within the CNS, if distributed evenly the concentration would be thirty times that seen in the brain after IV administration of a similar quantity. These assumptions are crude and unlikely to be correct: equilibrium may not exist, ICV clonidine is not evenly distributed within the CNS and the amount of clonidine present in the brain after IV administration are not derived from thse experiments. However it is likely that the concentration ratio ICV/IC greatly exceeds 1.5, the hypotensive potency ratio ICV/IV. Even including peripheral hypotensive mechanisms the two ratios remain unreconcilable and the CNS concentration seen with ICV administration is not reflected by the magnitude of the hypotensive effect and the areas of the brain reached with ICV administration are not those acted upon by IV administered clonidine. An action of IV clonidine on sites near the area S or floor of the fourth ventricle is unlikely and these areas not involved in the usual response to clonidine.

This conclusion probably applies to other experiments involving ICV administration and at the least suggests that their conclusions ought to be viewed cautiously. Very different plasma concentrations of clonidine follow IVert administration. The reduced rate of fall of plasma concentration between 0 and 1 min reflects the slower rate of administration used with IVert, given over 40 seconds, but the later slow decline indicates a progressive release from the brain. Porsius & Van Zwieten (1978) compared the plasma concentration of ^{14}C nicotine after IV and IVert administration. Immediately following IV injection the plasma concentation was high and declined rapidly, after IVert injection this peak did not appear suggesting that first pass uptake into the brain occurs. A slow release from a depot in the brain would account for the abscence of a peak plasma concentration. The potency of the IVert route and the autoradiography results indicate that first pass uptake into the brain tissue is high. The maintenance of the hypotension and

bradycardia shows that release from the brainstem is not rapid. Easy access put poor egress is hard to explain. Clonidine is lipophilic and would in consequence move readily into the brain during the first pass when local plasma levels are likely to be very high but as the plasma level falls so CNS clonidine concentrations should follow those of the plasma. The recovery from IVert clonidine is more rapid than that following IV administration, see previous chapter. Clonidine is not as lipophilic as thiopentone, a barbiturate anaesthetic, which rapidly leaves the brain after IV bolus administration. It initially achieves a high CNS concentration because the brain has a high blood flow, measured in ml/gm tissue, and its effective partition coefficient is 2000. That for clonidine is between 3 and 9 therefore movements across the blood brain barrier will not be as rapid. Pentobarbitone and antipyrine have effective partition coefficients that straddle that of clonidine. Their penetration half times, from plasma to brain, are 4.0 and 5.8 mins. If this carries over to clonidine and is applicable to movement out of the brain the recovery from IVert clonidine appears reasonably in line with its lipophilicity. Initially during the first pass the plasma/CNS concentration ratio will be very large, equilibration will not occur but some clonidine will reach the brain. As plasma levels fall the concentration ratio CNS/plasma will be smaller than that initially moving clonidine into the brain and movement will in consequence occur more slowly and the recovery from the hypotensive actions appear slowly, probably in line with brain concentration.

The plasma levels of tritium after IV administration of ^{3}H clonidine declines rapidly over the first two minutes, after administration continue to decline but more slowly between two and ten minutes and subsequently stabilize. The brief hypertensive episode corresponds with the peak plasma concentration suggesting that this is a peripheral action. Clonidine given by the ICV route escapes very slowly from the CNS probaby achieving concentrations greatly in excess of those seen after an equipotent IV injection. In consequence the sites of ICV hypotension and bradyardia are different from those involving IV clonidine, this excludes the area T1
S and the floor of the fourth ventricle as sites of hypotensive

action for IV clonidine. IVert clonidine reaches the brain during the first pass and subsequently slowly leaves the areas reached. The decline if plasma concentration is slower than with IV which may reflect continual release from a depot established during the first pass.

Plasma Tritium Concentration CPM/Sample

IV Clonidine

Rat No		1	2	3	4	5
Time	0.25	3485	2247	1888	3129	3162
mins	0.50	1360	1020	1576	1111	1110
	1.0	916	786	1090	771	830
	2.0	714	613	782	590	661
	5.0	670	579	671	673	626
	10.0	571	560	638	550	510
	20.0	567	515	593	507	473
	40.0	575	475	610	506	489
	60.0	581	513	632	550	452

Mean Arterial Pressure mmHg

Time	-5	82	101	102	108	104
mins	-2	80.5	102	102.5	104	103
	-1	81	100	102.5	104	103
	0.25	108	145	122	145	131
	0.5	73	100	98	120	103
	1.0	72.5	89	93	97	92
	2.0	68	87	89	96	85
	5.0	65.5	74	87	96	83
	10.0	63	69	86	92	82
	20.0	63	72.5	85	92	76
	30.0	63	72	87	96	77.5
	40.0	66	74	85	100	77
	50.0	70	76	87	99	75
	60.0	70	76	87	99	75

		ICV Clonidine DPM			IVert DPM
		...1.....2.....3...			
Time	0.5	27	1	-2	2617
mins	1.0	257	248	229	2753
	2.0	267	265	235	1831
	4.0	245	237	201	1270
	6.0	—	—	—	809
	8.0	214	224	201	763
	10.0	—	—		567
	12.0	220	224		830
	14.0	—	—		479
	16.0	226	234		507
	20.0	213			
	25.0	218			
	30.0	215			

* The DPM figures given are calculated from the quantities of tritium given and have been normalised for 6 uCi/animal and 10 ul plasma samples.

Conclusions And Suggestions For Further Work

The inactin anaesthetized rat is a useful model for cardiovascular research, it maintains a stable blood pressure and heart rate, shows reflex responses to asphyxia, vasodilator and vasopressor drugs and responds to clonidine with a reduction in heart rate, peripheral resistance and mean arterial blood pressure. The initial reduction in peripheral resistance occurs through a reduction in sympathetic nerve tone, shown by a novel hindlimb perfusion designed to separate acute nervous and blood born responses. This showed that clonidine exerted a central action in this preparation as the reduction in sympathetic vasoconstrictor activity outlasted the brief pressor response to IV clonidine. The clonidine induced reduction in heart rate involves sympathetic tone to the heart and no evidence was obtained for resting vagal tone or clonidine induced bradycardia involving vagal tone.

Clonidine was administered by four different routes IV, IC, ICV and IVert and the intensity and time course of the responses compared. IV administration leads to a rapid dose dependant reduction in both heart rate and blood pressure. Responses with IC injection are similar to IV. ICV is slightly more potent in lowering both heart rate and blood pressure but the effect appears slowly, peaking at 10-20 minutes. IVert is some twenty times more potent than IV. The onset of activity is fast and the recovery more rapid than that seen after IV.

A comparison was made of the distribution of clonidine after each route of administration to establish potential sites for the hypotensive and heart rate reducing actions. IV administration resulted in an even distribution throughout the brain. IC was concentrated in rostral brain areas. ICV to areas bordering on the ventricular sytem including the spinal cord. IVert, the most efficacous route, resulted in high concentrations in the medulla, pons, medulla spinalis and areas slightly rostral to these locations. This clearly shows that the vertebral arteries are important in delivering blood to the brainstem. The autoradiographic experiments and measurement of plasma concentrations clearly indicate that, when given ICV, clonidine reaches concentrations in

parts of the brain far in excess of those achieved with IV administration. In consequence it is proposed that ICV injection is not lowering blood pressure and heart rate by the same mechanism as IV clonidine. This implies that the area S and floor of the fourth ventricle are not the sites of action involved in the clinical response to clonidine. Clonidine IVert is active in doses likely to achieve similar concentrations in the brainstem to those found after IV administration. The prior exclusion of areas bordering the ventricular system shows the site of action to lie within the medulla or pons but its location is not readily reached from the ventricular system.

It is possible that no one site of action explains the cardiovascular effects of clonidine. The inability to locate a single site would confirm this but as yet unsufficient data exists.

This study clearly indicates that the use of ICV administration to deliver drugs to the brain, bypassing the blood brain barrier, does not provide equal access to all areas of the CNS. This techique has been widely employed often without sufficient precautions with regard to spread of drug and local concentration.

Further Work

The pursuit of the site of action of clonidine with local injection and autoradiography appears promising. It would be useful in clarifying the role of the area S and floor of the fourth ventricle as sites of action. Topical application of ^{3}H clonidine would be expected to reveal concentrations greatly in excess of those seen with IV administration. Similarly the hypothalamic and spinal sites could be readily investigated. The study would then require microinjections of suitably small quantities of clonidine into likely central sites. Likely areas would include those containing alpha 2 adrenoceptors and areas associated with cardiovascular control. A method of locating potential sites would be to use retrograde staining techniques to follow autonomic efferents into the CNS, then repeat the retrograde work from the sites previously identified, in practice mapping cardiovascular pathways. Using autoradiography to establish the resulting distribution and concentration. That the hypotensive effect could be achieved, with local concentrations in the range seen with IV

administration, would be taken as the definitive test of putative sites of action. The study would then proceed with intracellular microelectrode examination and iontophoresis to establish the pharmacological requirements for action at the site.

An autoradiographic study on the distribution of histamine H2 antagonists after ICV administration would be interesting. Indicating the areas of the brain required for the reduction in the hypotensive effect of clonidine and the concentrations achieved. The latter is of interest in establishing whether the histamine H2 antagonists are present in concentrations associated with histamine antagonism or whether another action is involved. Further studies with cats might establish the reasons for the failure to show antagonism in this species. A possible cause might be alterations in the distance from the ventricular surface of the relevant site of action occuring with increased brain size.

The work of Zaimis on peripheral hypotensive actions of clonidine should be pursued particularly with respect to longterm changes in vascular reactivity. Chronic administration could be managed with clonidine added to drinking water and the development of hypotension followed with tail cuff blood pressure measurements. Comparison of isolated tissue responses to nerve stimulation and exogenous drug application could be used to chart alterations in tissue reactivity. The efficacy of treatments too low to alter arterial blood pressure might provide an insight into the anti migraine properies of clonidne, they appear almost uninvestigated. They seem to utilise quantities of clonidine below the hypotensive threshold.

Clonidine has a peripheral action on presynaptic receptors but their importance has been questioned. The lack of peripheral vasoconstictor effects mediated through alpha 2 adrenoceptors is surprising and deserves investigation. Peripheral alpha 2 adrenoceptors have been located in the vasculature and mediate vasoconstriction. Clonidine is believed to act centrally on the same type of receptor. It should follow that hypotenisive and hypertensive actions would appear over the same dose range. However higher concentrations appear to be required for peripheral vasoconstriction. A plausible approach could involve perfusing a neurally intact hindlimb with a perfusate containing clonidine. Both

changes in hindlimb vascular resistance to stimuli evoking reflex alterations, carotid occlusion, asphyxia vasoactive drugs, and changes in the resting resistance could be investigated. A further development would require cross perfusing the limb from a second animal and administration of clonidine to the donor. A comparison between the hypotensive effect and alterations in the responsiveness of the hindlimb to reflexes causing changes in vasoconstrictor tone would highlight peripheral and central effects.

Quantitative autoradiography could be employed in catecholamine turnover experiments to increase their spatial resolution, by loading with tritiated noradrenaline and looking at the rate of decline during various manipulations. If care was taken to control afferent inputs during clonidine treatment the effects on different catecholamine containing nuclei could be investigated.

Finally further work on the differential effects of clonidine on autonomic efferents would be interesting, particularly the action on muscle vasodilator nerves and different vagal fibres. Clonidine lowers vagal drive to the stomach but increases cardiac vagal tone.

"Pulseless" Roller Pump

Roller pumps are inherently pulsatile. They operate by occluding flexible tubing and as the roller rotates fluid is pushed forward. The rollers and the race, the area supporting the tubing when it is in contact with the rollers, are so designed that at least one roller always occludes the tubing. Occlusion is sufficient to maintain different pressures on either side of the roller. Pulsations arise from a change in the rate of fluid delivery. The major cause is roller engagement and disengagement. As a roller disengages the tubing returns to its original dimensions. The increase in volume briefly reduces the forward flow of fluid and a drop in pressure downstream of the pump results. Engagement of a roller has the opposite effect upstream of the pump.

In cardiovascular physiology roller pumps are often used to perfuse a isolated limbs or organs and the pressure generated downstream of the pump is taken as a measure of resistance. When the pumping rate is constant a change in pressure does represent a change in resistance but roller pumps are pulsatile. The size of the associated pressure change depends upon the compliance of the system. However tissues and receptors respond differently to pulsatile and non pulsatile pressures (Cox & Bagshaw 1980, Spickler at al 1967) making non pulsatile or controlled pulsatile perfusions desirable. A common method of reducing pressure pulsations is to increase artificially the compliance of the system using a side arm containing air. The column of air changes volume during pulsations damping them, the degree of damping depending on the volume of air in the side arm. Damping reduces the frequency response of system which is undesirable and makes the experimental imposition of pulsations more difficult. However if the compliance of the perfused tissue changes, but not the resistance to flow, the size of any pressure pulsation also changes thereby altering the conditions of the perfusion. A balance has to be struck between frequency response and the accepted level of pulsation. Clearly a reduction in the inherent pulsatility of roller pumps used for circulatory perfusion experiments is desirable.

Potential Causes Of Pulsations

1) Engagement and disengagement of rollers.

As mentioned above the rollers cause pulsations, the volume involved depends upon the diameter of the roller and the degree to which the tubing is compressed. The magnitude and duration of the pressure pulse further reflects time over which the volume change occurs. If rapid the pulse will be brief but intense and if prolonged smaller but of greater duration, an inverse relationship. It follows that variations between rollers will cause different pressure pulses. Examination of the Watson Marlow pump (MRHE 200) shows variations in the diameter of rollers and variations in the distance from the centre of rotation which lead to the generation by each roller of a different sized pulse.

2) Tubing not lying in the plane of roller rotation.

As the roller moves it pushes fluid ahead. The volume displaced by a given rotation is the length of tubing under the roller X the internal diameter. Therefore if the tubing is at an angle to the plane of rotation of the rollers the volume pumped is not the distance moved by the roller multiplied by the internal diameter of the tubing. It becomes an function of the angle from the plane of rotation. If the tubing is free to move the angle will alter unpredictably and alterations in flow arise.

3) Changes in rate of pumping.

A change in the speed of the pump will cause a change in fluid output. This may arise during one cylce of engagement, pumping and disengagement if the torque of the motor is insufficient. In the Watson Marlow pump this occurs at low speeds during engagement of the roller with the tubing.

Solutions

1) Removal of differences between rollers.

Ideally the rollers should be located symmetrically around the centre of the motor spindle but in practice symmetry is achieved around the centre of the roller holder. In the Watson Marlow pumps the roller holder is attached to the motor spindle by a grub screw.

When tightened this moves the rollers relative to the motor spindle and the symmetrical arrangement is lost. A new roller holder that attaches to the motor spindle by friction was made, this preserves symmetry by ensuring that the centre of the roller holder and the spindle are the same.

New rollers were made from brass to high tolerances and their effective diameter measured during rotation on the motor spindle. The effective diameter is the distance from the centre of the motor spindle to the edge of the roller. This is not the distance from the centre of the motor spindle to the centre of rotation of each roller plus half the diameter of the roller because this omits any difference between the individual roller spindle and the centre hole of the roller which reduces the effective size of the latter. Differences between rollers in postion on the pump were $\pm$ one thousandth of one inch.

2) Alterations to the race.

If disengagement of the roller from the tubing occurred slowly with a constant rate of change in volume of the latter pulsations from this cause would be eliminated. The race was redesigned accordingly.

This requires that the disengagement arc is equivalent to the pumping arc. In the Watson Marlow as one roller disengages the following roller is fully engaged, this is required for the maintenance of different pressures on each side of the pump. It was decided to make operation of the pump "pulseless" for engagement and disengagement. The new design has a roller leaving the race for the whole of the pumping arc, therefore the following roller has to be in contact with the race to maintain the seal. When one roller has just completely left the tubing the following roller is about to start disengaging and the next roller must be fully engaged. In turn its following roller is beginning to engage. Four rollers are then close to the tubing. This is difficult to arrange with three rollers as the pumping arc is then 1/3 each rotation and the required disengagement and engagement arc 2/3 of a rotation of the motor spindle. A six roller pump was decided upon, giving a roller tubing contact arc of 1/2 a rotation. Increasing the number of rollers leads to more pulses per rotation but reduces the size of the race, making construction easier. Increasing the number of rollers

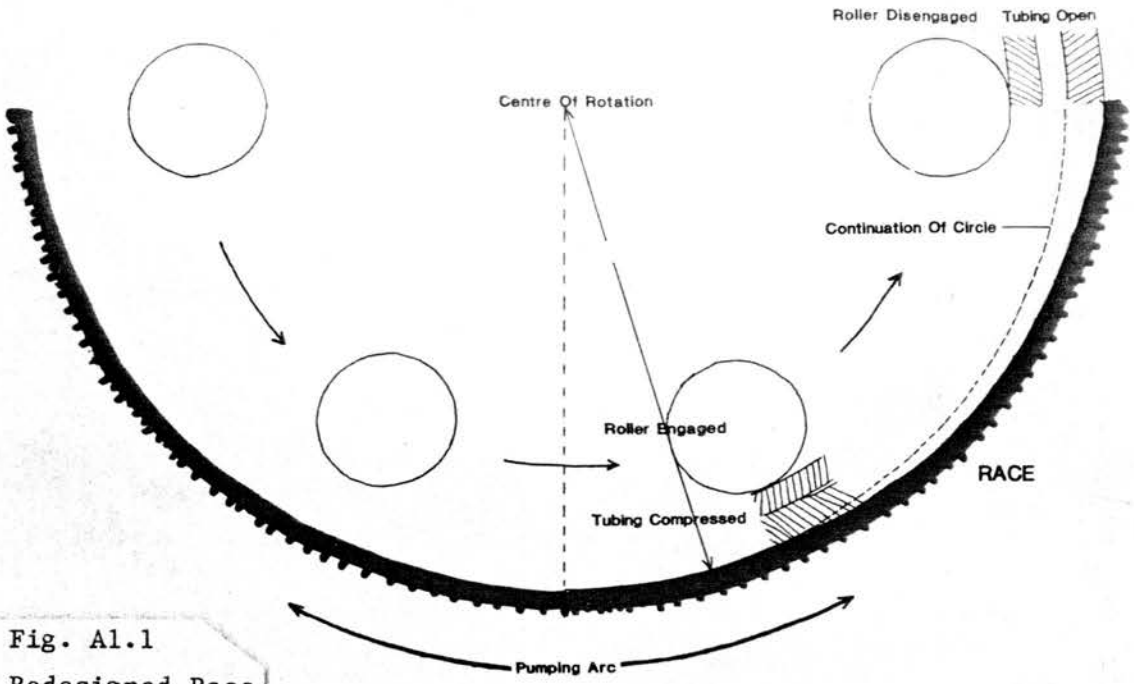
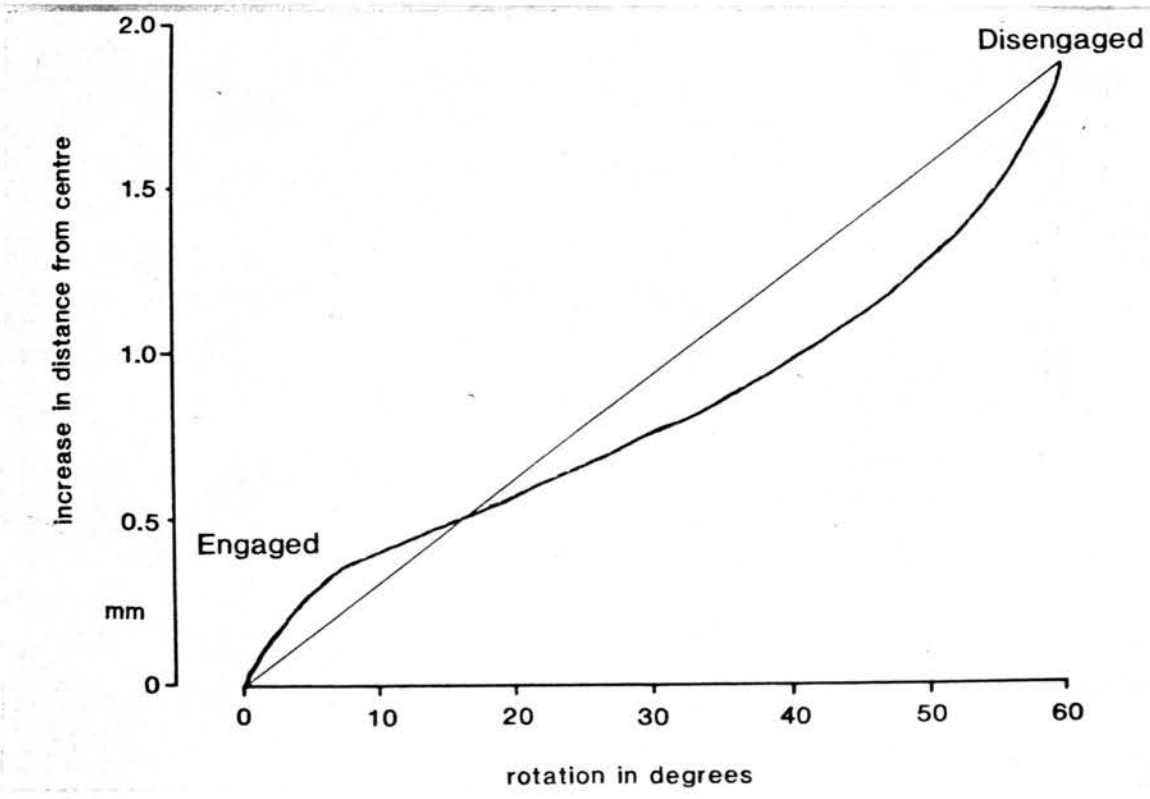


Fig. A1.1
Redesigned Race

a. In the new race the rollers disengage over 1/6 of a complete cycle, similarly pumping occurs over 1/6 of a cycle.

b. Graph showing the increase in the distance, from the centre of rotation, of the race during the disengagement cycle. It is not linear, see line on graph, and is intended to produce uniform change in the volume of the tubing throughout the cycle.



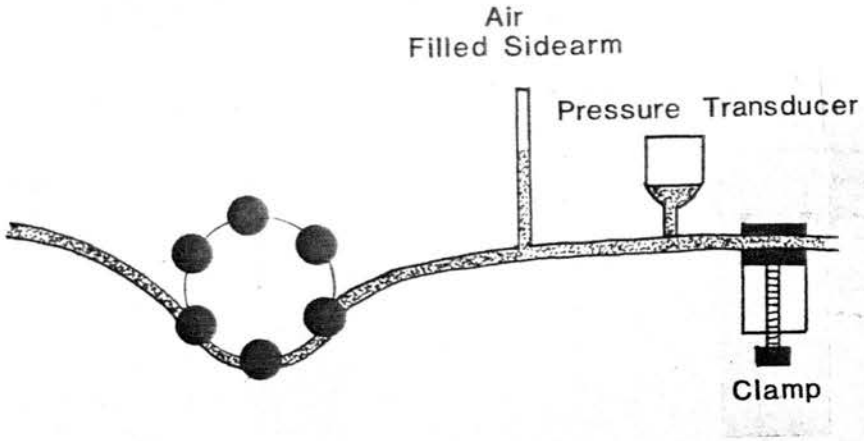


Fig. A1.2

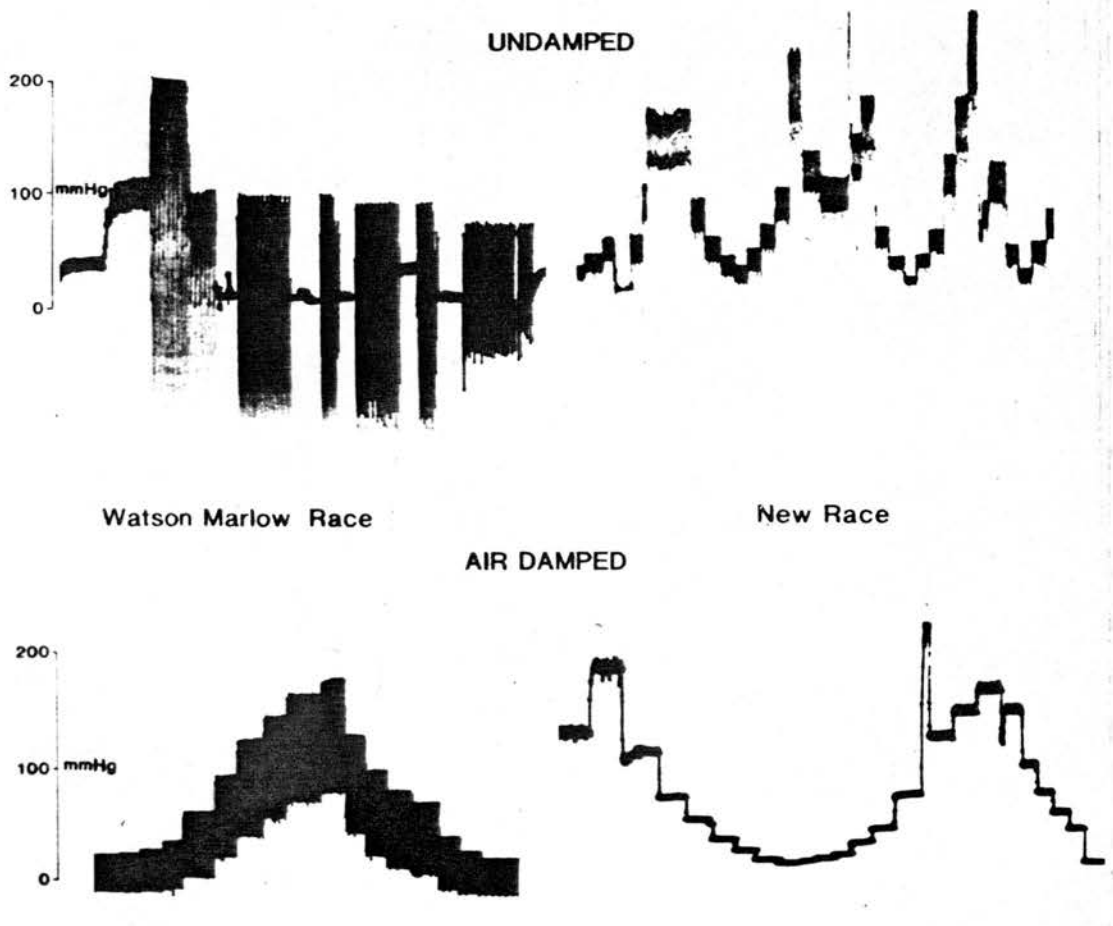


Fig. A1.3

Fig. A1.2

Test Arrangement.

Pump with pressure transducer and clamp downstream. The clamp is used to increase the resistance to flow of the tubing and permit the development of pressure.

Fig. A1.3

Operation Of Old And New Systems.

Set up without damping the old, Watson Marlow race and rollers produce large changes in pressure downstream of the pump. These range from below 0 mmHg to above 200 mmHg, both are off scale, when a mean pressure of 95 mmHg is developed. In the top left trace the solid parts of the trace show the mean pressure developed, recorded with the preamplifier set to mean, The pressure pulses to their left occur in the same circumstances exccept that the preamplifier was operating undamped. To keep pressures positive the sytem was damped with an air filled side arm between the pump and transducer.

The new design is displayed in each mode and illustrates the advantages of the redesigned system.

The traces were all made with the same pump speed and different pressures were produced by altering the resistance in the outflow tubing.

employed makes uniformity of construction more difficult. Six was accepted as a compromise between the size of race and number of rollers.

Redesign of the race

A micrometer was used to push a roller against the tubing, reading the micrometer gave the movement of the roller. Changes in the internal volume of the tubing were measured by displacement of water along a thin bore polythene tube attached to the roller tubing. This allowed accurate measurement of volume changes. The relationship between volume displaced and roller movement was used to redesign the race. The new race was intended to cause uniform changes in internal tube diameter throughout the engagement and disengagement cycle, Fig A1.2a,b.

Redesigned Rollers

The diameter of the rollers affects the volume displaced within the tubing. Similarly the greater the internal diameter of the tubing the greater the volume displaced by a given size of roller.

A small roller with small bore tubing would lead to a small displacement of volume. With the unaltered race where disengagement is rapid a small pressure pulse would result. However the volume pumped by one rotation would be reduced, requiring a higher pump speed. In consequence the volume change would occur over a shorter period, tending to negate the advantage. A redesigned race would reduce the rate of change of tube internal volume. The degree of pressure damping required to cope with a large number of small pulses is less than that required for a smaller number of large pulses, depending upon the size of pulse not their frequency.

The decision about tube size was determined by the particular pump available. The range of speeds over which it produced a constant speed of rotation and the range of pumping rates required forced the adoption of 1.6 mm internal diameter tubing.

A roller size of 6.35 mm was adopted. As the diameter of the roller is reduced the volume that it displaces on contact with the tubing does fall in a linear manner. The size chosen is smaller than that used by Watson Marlow and is a convenient size for machining.

To reduce lateral movement of the tubing the rollers were provide with flanges, limiting the tubing to a groove just wider

than its external diameter.

Measurement Of Pressure Pulse During Pumping

The output of the pump was attached with a sidearm to a pressure transducer and the tubing constricted downstream of the transducer with a clamp, Fig A1.2. The clamp restricts fluid outflow and allows generation of pressure changes. The test system has no damping except that provided by the elasticity of the tubing. Switches between the Watson Marlow race and rollers and the altered equipment were made without altering the clamp.

Results

The new race and rollers are a marked improvement over those supplied by Watson Marlow but have not entirely eliminated the problem of pulsations, A1.3. The variations between individual rollers has been reduced but still exist, the residual pulse associated with disengagement is different for each roller.

Disadvantages

The race is specific for one size of tubing, internal and external diameter are involved in the design. A range of races are required if a variety of tubing is to be employed. Slight variations occur in different batches of tubing, degrading the operation of the race.

Further Possible Improvements

The rollers and their housing were made to the highest tolerances feasible in the departmental workshop and further improvements in this direction are unlikely.

When adjusting the race, varying the distance from the rollers to achieve the minimum pulse, a critical point is reached where further movement away results in a drop in pressure with some rollers. Due to failure of the engaged roller to sufficiently compress the tubing and make a seel. This occurs when the pulsation associated with other rollers is positive. Increasing the compression of the tubing by the roller might effect a reduction, redesinging the race.

The use of a roller pump with a lower speed, permitting the use of wider bore tubing. This would enable easier construction of the

race, with 1.6 mm tubing the cutting equipment was operating at its limits.

In use the tubing does not lie unmoving against the race but is stretched by each roller as it passes and recoils afterwards. The design of the race was based upon a static model of roller tubing interaction and did not take this into account. Reducing the friction between the tubing and roller might reduce this problem.

Reducing the diameter of the rollers. The volume displaced by a roller when opposed to the tubing is a function of the the its diameter. This is a possible approach and would necessitate buildind new rollers and a new race.

The type of tubing may be of importance, its compliance. Soft tubing will be stretched by the rollers and recoil to a greater extent than stiff tubing. However the conctact of a roller on stiff walled tubing leads to a greater displaced volume.

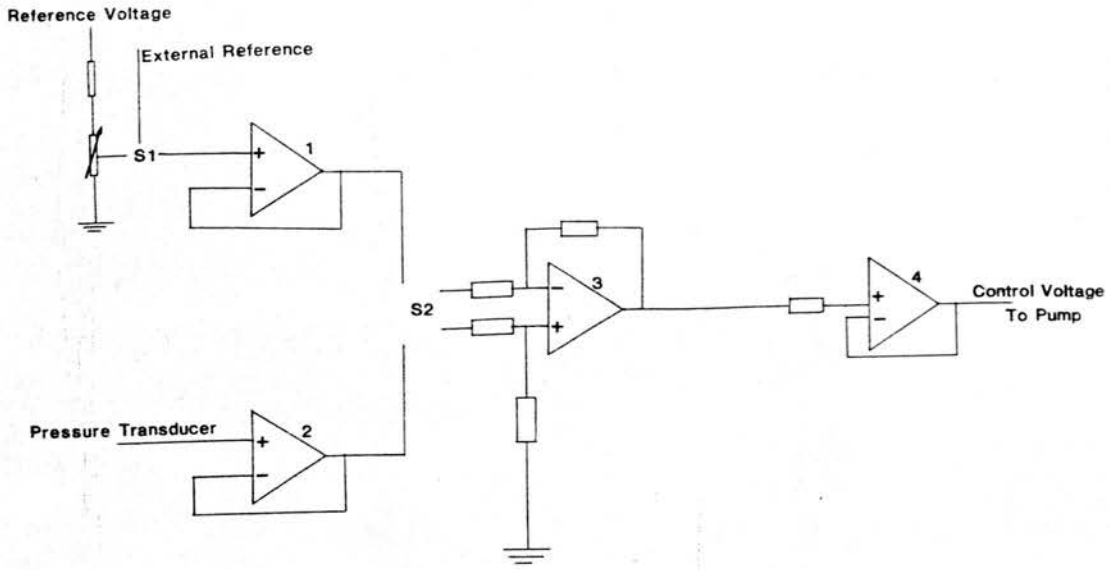


Fig. A1.4

Circuit Diagram Of Control Box.

In essence a differential amplifier with buffered inputs from the transducer and a reference voltage. The amplified difference between the two determines the control voltage which in turn alters the pump speed, which tends to reduce the discrepancy between them.

Switch S1 selects either an internal or external reference voltage. Switch S2 alters the polarity of the differential amplifier output. This enables the use of the system to infuse vasodilating drugs, when the measured blood pressure exceeds that set by the reference voltage the rate of infusion is increased, with a vasoconstricting drug the reverse is required.

The amplifiers are standard 741 operational amplfiers, powered from $\pm$ 15 volts, not shown on diagram.

The gain of the differential amplifier was set to 400.

Feedback Controller For Watson Marlow Roller Pump

The speed of the pump can be altered by altering the input voltage on a socket at the rear of the machine. Therefore by taking the pressure from a transducer downstream of the pump and passing it through a differential amplifier the pressure generated may be controlled a the second, reference voltage, input to the amplifier, Fig A1.4. The greater the difference in the two voltages the greater the amplifier output and the faster the pump runs. The gain of the amplifier determines how closely the pressure generated follows the second input to the amplifier. If the pressure at the transducer alters the feedback loop operates to return the pressure the original level.

The output voltage of the feedback controller varies from 0-14 volts.

Uses Of System

1) Infusion of drugs.

The pump was attached to a solution of either glyceryl trinitrate or phenylephrine and connected to a vein. The pressure transducer measures arterial pressure, Fig A1.5a. The system was intended to provide maintained changes in blood pressure by using the feedback loop to alter the rate of infusion of a vasoactive substance. Altering the reference voltage selected the pressure to be achieved, A1.6c.

The system worked sometimes but the fast reponse time of the feedback system and the slower response of the animal to changes in infusion rate led to oscillations in pressure and infusion rate. A fall in pressure leads to an increase in the rate of infusion which after a delay raises pressure which in turn reduces the infusion rate leading to a drop in pressure etc. Further at any time only a vasodilator or vasoconstrictor can be used, restricting changes in blood pressure to increases or decreases.

A reduction in the response time of the feedback loop with large capacitors on the output of the differential amplifier reduced the oscillations.

2) Constant pressure source

Leaving the reference voltage unchanged makes the system operate as a constant pressure source for use in perfusions, Fig A1.5b. Changes in resistance are shown by alterations in the control voltage driving the pump, A1.6a.

3) Variable pressure source

Fig A1.4c. The input to the pump was attached to the cardiac end of the common carotid artery and the output to the central end of both common carotids. The head and baroreceptors receive a pressure determined by the control voltage, A1.6b. The intention was to investigate changes in heart rate associated with blood pressure at the brain and baroreceptors. With rises in pressure heart rate falls and so does blood pressure recorded from the femoral artery. The surgery involved is not too onerous and the preparation was intended to supercede studies on heart rate where IV vasoconstrictor or vasodilator drugs are used to alter afferent input and evoke a reflex response.

Once a "pulseless" roller pump that required little pressure damping had been made a signal generator could replace the reference voltage, permitting the generation of static and dynamic pressures at high frequency.

4) Flowmeter

If a pressure transducer downstream of the pump has its output fed into the reference voltage input of the differential amplifier the pressure generated downstream will follow that upstream of the pump, Fig A1.4d. The control voltage to the pump then becomes a measure of pumping rate or blood flow of a tissue perfused perfused tissue. A solution for those not having sophisticated flow meters.

This approach has not been used and is therefore speculative.

At the time when this system was being considered Mohram published a paper (1980) which has many similarites. The pump used has four rollers and is designed to reduce only pulsations downstream of the pump. The size of tubing used, 0.25 inch, makes manufacture of a race easier but appears to preclude use with small animals.

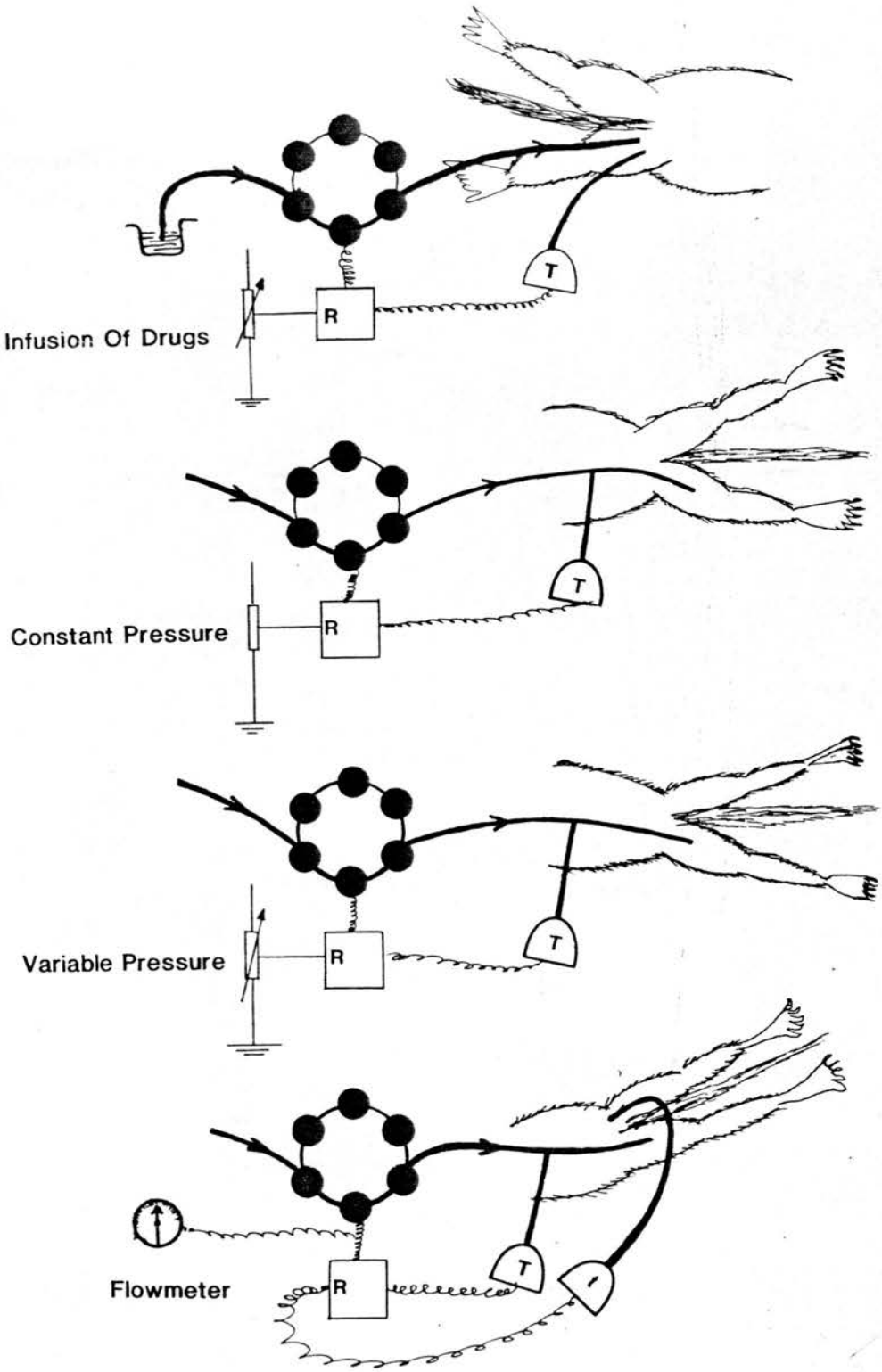

Infusion Of Drugs
R
T
Constant Pressure
R
T
Variable Pressure
R
T
Flowmeter
R
T

Fig. A1.5

Applications Of The Control Box.

Illustrated by different applications in an animal hindlimb.

T - pressure transducer

R - reference voltage input in the feedback controller.

a) Infusion of drugs.

When the blood pressure falls below the level set by the reference voltage the pump is activated and a vasoconsting drug infused. To operate with vasodilating drugs the inverting switch in the feedback controller must be activated, the pump is activated when blood pressure rises above the required level.

b) Constant pressure perfusion.

Alterations in vascular resistance are recorded by the pressure transducer and the feedback controller alters the pumping rate accordingly.

c) Variable pressue source.

As in b) except that the reference voltage is varied which in turn changes the resulting pressure.

d) Flowmeter.

t, a second tranducer, measures arterial pressure which is used as the reference voltage. Thereby causing the perfusion pressure to follow. The control voltage driving the pump is a measure of the pumping rate and therefore perfusion rate at the ambient arterial pressure. It acts as a flowmeter. The system has not been used in this mode.

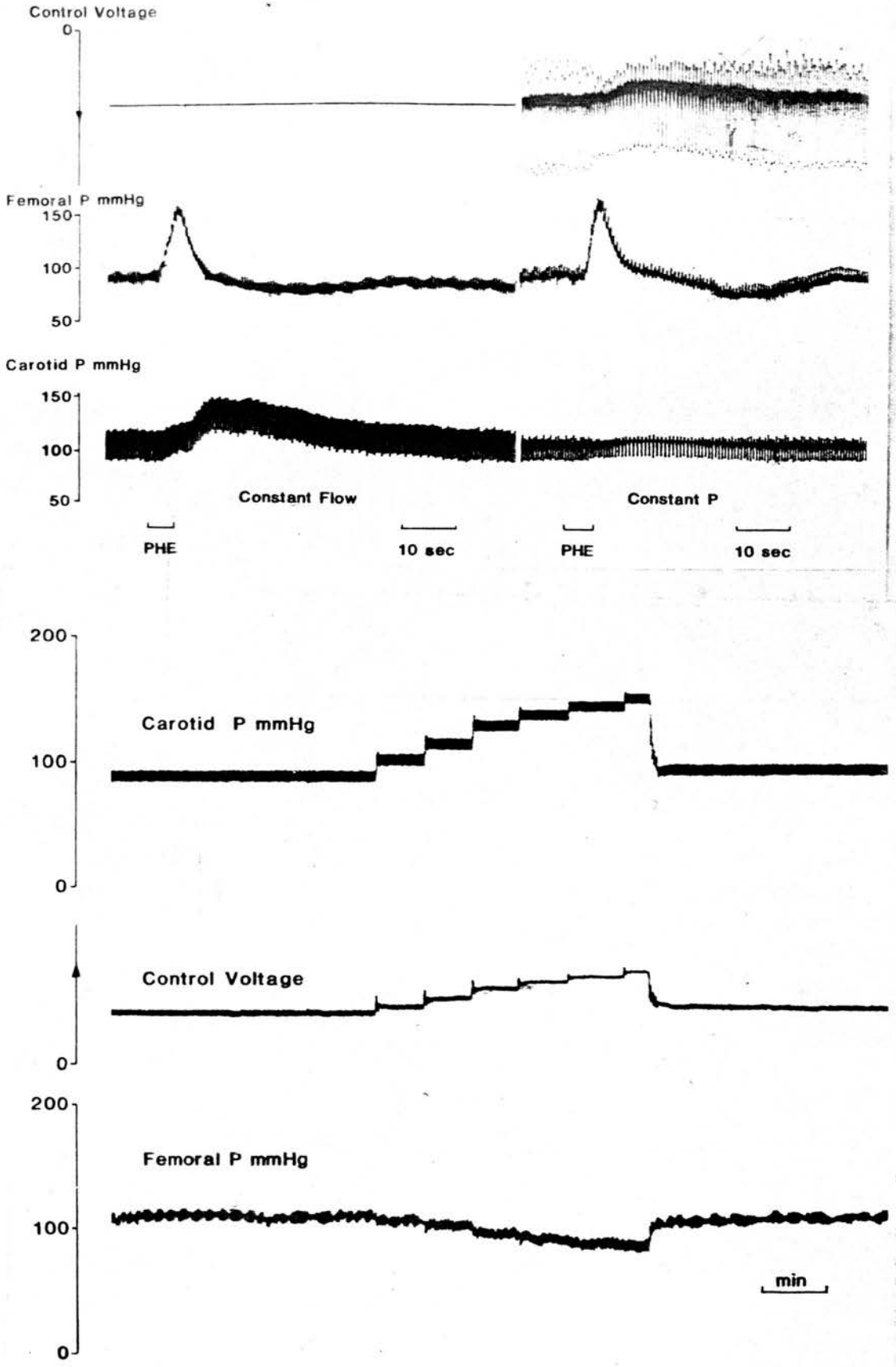

Control Voltage
0
Femoral P mmHg
150
100
50
Carotid P mmHg
150
100
50
Constant Flow
Constant P
PHE
10 sec
PHE
10 sec
200
Carotid P mmHg
100
0
Control Voltage
0
200
Femoral P mmHg
100
0
min

Fig. A1.6
The feedback controller in operation

a) The panel on the left has the pump operating at a constant rate perfusing a carotid artery. IV PHE increases arterial pressure and after a delay, accounted for by the volume in the external circuit, carotid pressure also rises. In the right panel the feedback controller is operating as a constant pressure source and no increase incarotid pressure occurs, instead the control voltage falls preventing the pressure rise. A pressure increase of 30 mmHg in the left panel is reduced to 5 mmHg.

b) Variable pressure source. The carotid artery is perfused and alterations in the reference voltage are used to change the carotid pressure. The control voltage increases with each pressure step and arterial pressure, recorded from the femoral artery falls. Arterial pressure changes are assumed to occur through the baroreceptors which were intact.

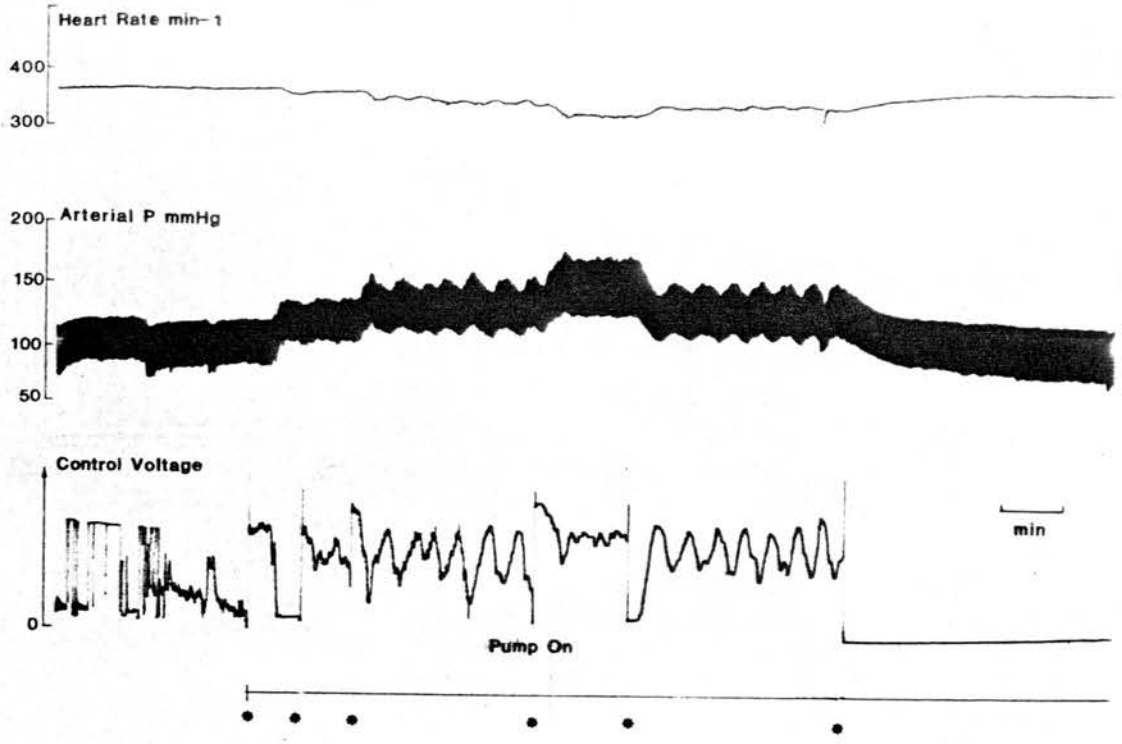


Fig. A1.6

c) Drug infusion.

PHE was given as a vasoconstrictor.

At the first * on the baseline the pump was turned on. Until then the changes in control voltage have no effect. At each subsequent * the reference voltage was altered, seen as a step change in the control voltage, either up or down. After the last * the controller was switched off.

Oscillations in arterial pressure are the major problem. A change in infusion rate in not immediately reflected in pressure changes and the delay allows oscillations to appear, further pressure does no rapidly drop when the infusio rate drops, t1/2 of PHE. The pump speeds up, pressure rises after a delay, pump slows attempting to reach a steady state infusion, but pressure overshoots the pump stops, infusion rate falls and the pump again speeds up which restarts the cycle. Slowing the rate of change in the control voltage should permit the attainment of a steady state. This arrangement is not applicable where acute pressure changes are required but is likely to be of use when steady pressures are required over a long period.

Neurophysiological Spike Discriminator And Counter

The neurophysiological spike discriminator was designed to separate action potentials from noise, distinguish between action potentials and measure frequency. Frequency is determined by counting the number of action potentials in a given period rather than the reciprocal of the interval between action potentials. The discriminator was intended to increase the yield from experiments involving nerve recording by permitting analysis of single units within a multiunit record. It differs from commercial equipment, which was not available, through the inclusion of a time window in the gating mechanism and the provision of a multiplexed output displaying the operation of the gate. Fig A2.1 shows a block diagram of the machine and fig A2.2 its operation.

Input/Gain

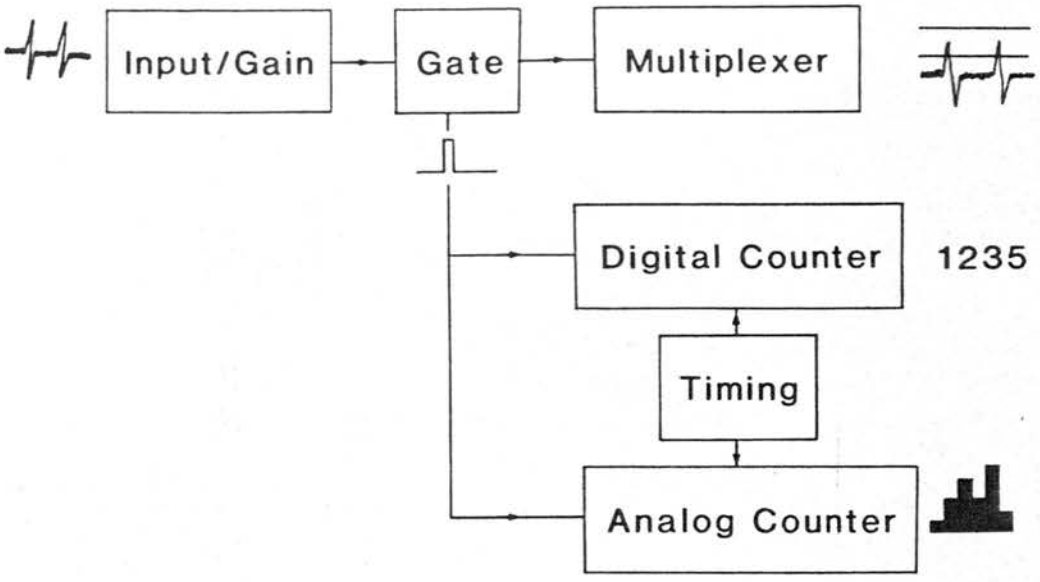
Provides a high input impedance, 2 M ohms, and a variable DC gain of X 1/2 - 15. Signals within the range ±14 volts are accepted at the input.

Gate

The gate consists of two reference voltages, V1 and V2, and a time window, T2. To generate an output pulse, P2, the incoming signal must exceed V1 but not V2 and recross V1 within the time window. When V1 is exceeded there is a period T1 within which an output pulse cannot be generated and a subsequent period T2 within which a pulse may be generated. This permits separate analysis of action potentials of different duration or amplitude. Fig A2.2-6 show the gate in operation.

Multiplexer

The multiplexer has a single output into which V1, V2 or signal are sequentially connected. As switching between inputs is at a high frequency the operation of the gate can be viewed on one channel of an oscilloscope. The switching cycle is V1-signal-V2-signal and the switching frequency 100khz therefore in 1 millisecond the signal is shown fifty times for 10 microseconds and V1 and V2 twentyfive times for 10 microseconds. Fig A2.8.


Input/Gain
Gate
Multiplexer
Digital Counter
1235
Timing
Analog Counter

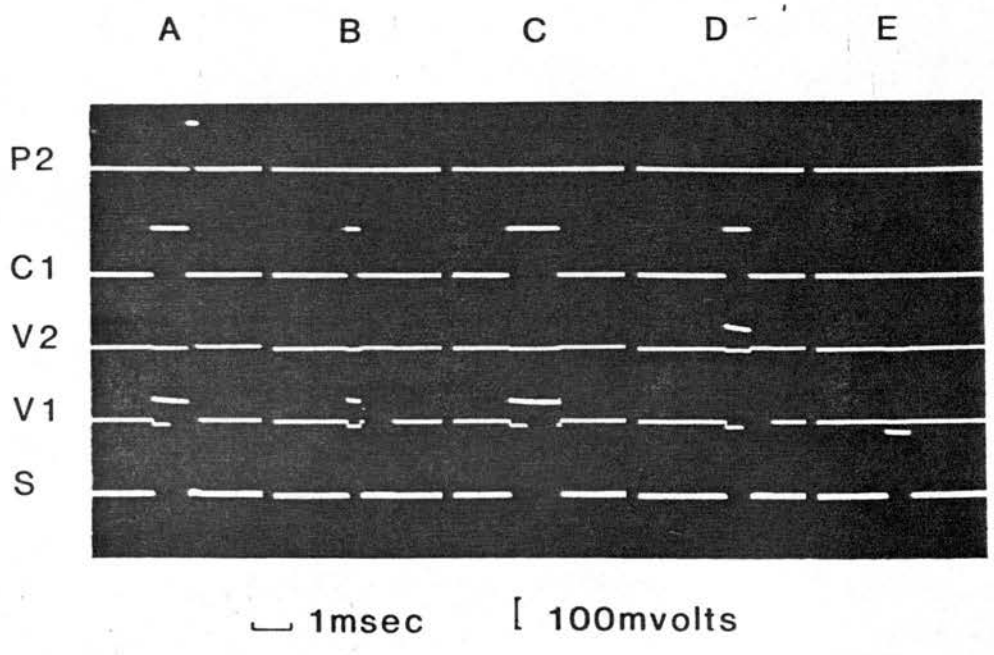

A
B
C
D
E
P2
C1
V2
V1
S
1msec
100mvolts

Fig A2.1 Block Diagram of Spike Discriminator

The gate is the central component, accepting the incoming signal from the input/gains stage and sending signals to the counters and multiplexer.

Fig A2.2 The Operation Of The Gate

A variable test pulse (S) is applied to the gate whilst V1, V2 and T2 and held constant.

S signal leaving the input/gain stage.

V1 lower reference voltage. The gap in V1 corresponds to the time gate T2. Hysteresis is apparent.

V2 lower reference voltage.

C1 output from the lower voltage comparator.

P2 output from the gate, goes high when the signal falls within the apperture set by V1, V2 and T2. It is initiated when S drops below V1. P2 is also apparent as a gap in the V2 trace.

Five separate traces are displayed A-E. They were taken from an oscilloscope.

A. S exceeds V1 but not V2 and falls within T2 and P2 is generated. note hysteresis in V1 and the gaps in V1 and V2.

B. S is shortened and falls outside T2 and fails to generate P2.

C. S is extended and falls outside T2 and fails to generate p2.

D. S exceeds V2 which prevents the generation of P2.

E. S falls below V1 and no output is produced.

In four of the five examples no output pulse appears.

During T2 the signal is displayed at the expense of V1 creating a gap in V1 corresponding to the time gate and similarly P2 switches V2 off in favour of the signal, indicating that an output pulse has been generated. Fig A2.9.

The gate may be made to appear static on an oscilloscope by using the output from the V1 comparator to initiate each sweep.

Analog Output

The number of output pulses from the gate are counted and passed into a ten bit, 0-1023, digital to analog converter giving an analog voltage output compatible with an oscilloscope or pen recorder. The analog output is updated at the end of each counting period, alternatively a ramp stepping once for each pulse and resetting to zero at the end of each period is available. Fig A2.9-10.

Digital Output

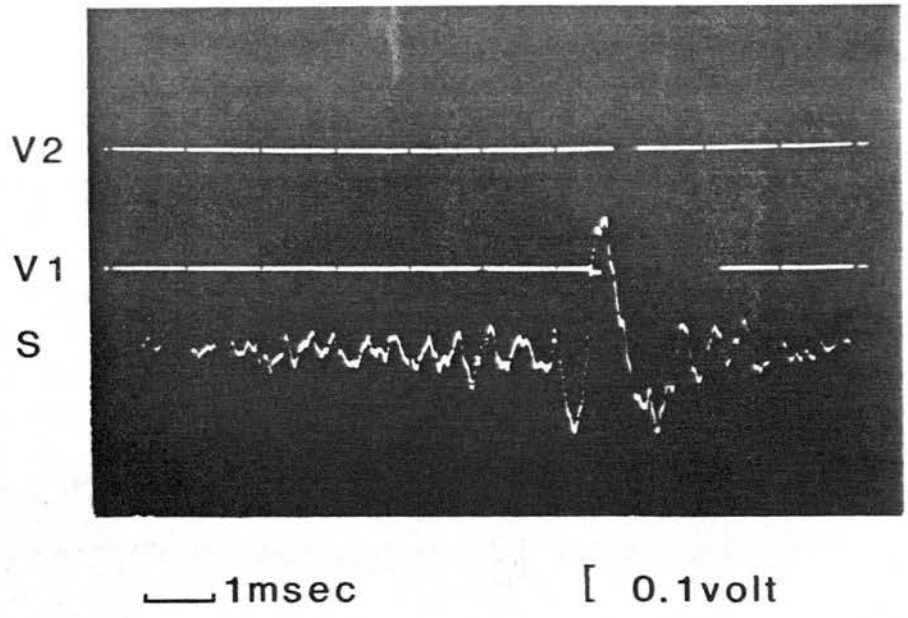
A four digit, 0-999, LED display gives a numerical count corresponding to the analog output.

Timing

An oscillator provides a wide range of counting periods, 0.1-10 seconds, and reference frequencies for calibrating the analog output.

Power Supply

The unit contains $\pm$15V and $\pm$5V D.C. stabilised power supply.



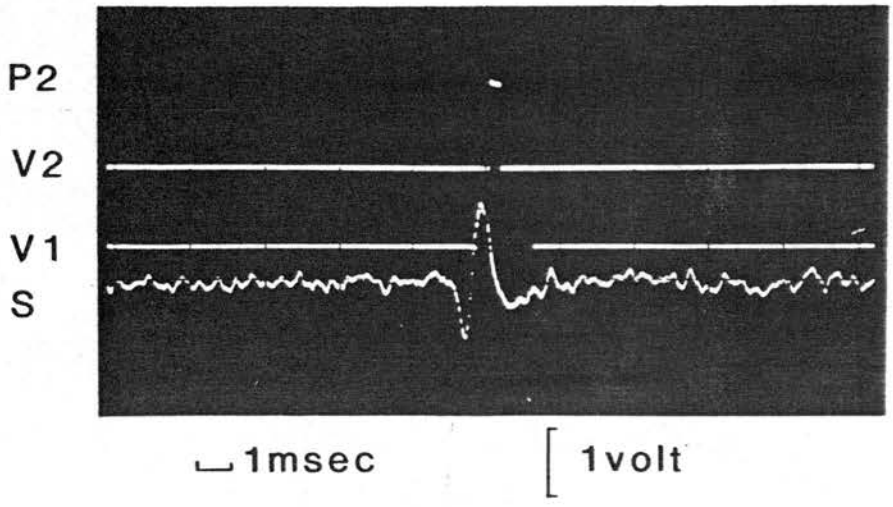


Fig A2.3-4 Operation Of The Gate With Action Potentials.

In each case the action potential falls within V1, V2 and T2 and an output pulse is generated. The time gate is apparent as a gap in the V1 trace and the gap in V2 indicates that a pulse P2 has been generated. In Fig A2.4 P2 is displayed .
The traces are taken from a storage oscilloscope.

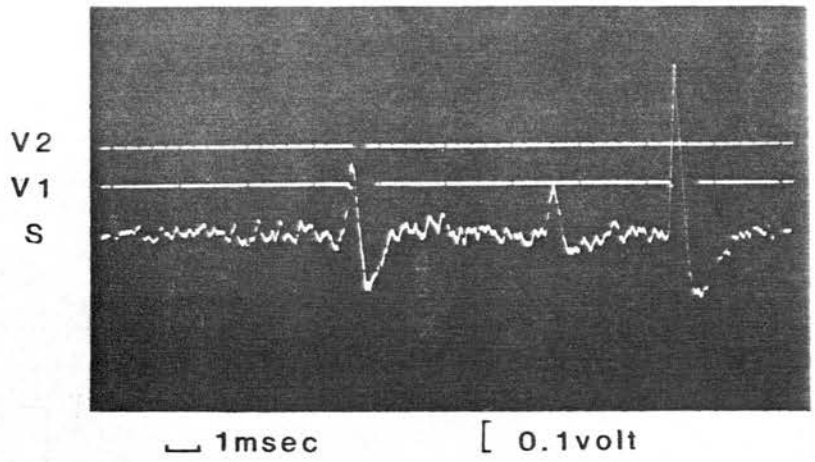


Fig A2.5 The Gate Operating With A Multiunit Nerve Recording.

The trace shows a multiunit record containing three action potentials of different height. Only the first falls between V1 and V2 and leads to the production of P.

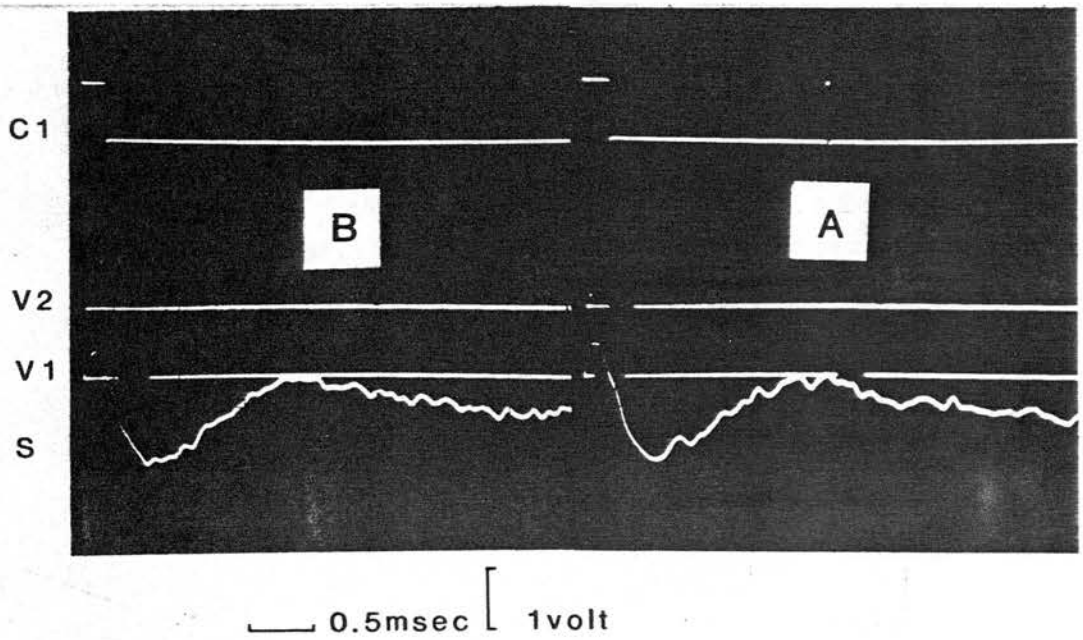


Fig A2.6 Operation Of The Time Gate With Action Potentials.

The same action potential is shown in A and B but in B the time gate has been extended, causing S to fall below V1 outside the time gate. Only in A is P2 generated.

The oscilloscoope sweep is triggered from the lower voltage comparator output C1. This means that in successive sweeps the postion of the gate on the oscilloscope remains unaltered.

Technical Details

This section explains the detailed operation of each portion of the apparatus and contains circuit diagrams. To make the circuit diagrams intelligible power supply connections and earthing have been ommitted.

Input/Gain

Components 3/4 348 A Quad 741 operational amplifier

Three 741 operational amplifiers are used to providing an input buffer, a variable D.C. inverted gain amplifier and an inverting unity gain buffer restoring the polariity of the signal presented to the comparators and multiplexer.

Gate

Fig A2.11

Components 2 x 74LS123 Dual monostable
74LS13 Dual schmitt trigger
74LS74 Dual D type flip flop
2 x LM748c Operational amplifier

The gate produces an output pulse when the incoming signal falls within the voltage and time windows set by the experimenter. When V1 is exceeded the output of comparator C1 changes and initiates a pulse from the monostable M1, the falling edge of which activates M2 whose pulse duration provides the time window T2. The period between V1 being crossed and the start of T2 and the duration of T2 are set using variable resistors on the front of the machine. When V1 is recrossed P1 is generated from M3 which in turn activates M4 if it is within T2 and V2 has not been exceeded. If V2 is crossed the D type flip flop is cleared, altering the output logic level which through the NAND gates prevents P1 reaching M4 and the generation of an output pulse, P2. T3 resets the D type flip flop as it simultaneously attempts to trigger M4 but the delay involved in the NAND chain prevents the reseting allowing M4 to be triggered if V2 has been exceeded.

748c operational amplifiers were used as comparators in

preference to LM710 or LM319 comparators as the expected maximum frequency of incoming spikes is in the kilo rather than megahertz range and the very fast response time of the comparators renders them prone to triggering from high frequency signals unrelated to action potentials. 74LS13 Schmitt triggers were used to increase the switching speed of the LM748s making a secure interface with TTL components. The C1 comparator incorporates hysteresis, this reduces the V1 after the incoming signal exceeds the V1 thereby preventing oscillation in the comparator output when the signal and reference are of similar magnitude.

The chain of NAND gates prevents P1 reaching M4 when the upper voltage comparator or time gate have been violated.

The 74LS123 monostables, used to generate the time gate, are retriggerable and the timing period starts anew whenever V1 is exceeded. This prevents triggering from a signal falling inside the time gate initiated by the preceeding pulse. The connection between M1 and the CLR input on M2 operates to terminate T2 if it is still active, making the time gate fully retriggerable.

The reference voltages, V1 and V2, are derived from potentiometers connected between earth and a 12 V zener diode stabilized supply.

An output from C1 is provided externally and is used to initiate sweeps of the oscilloscope displaying the gate, this produces a static display of the gate with action potentials superimposed. This is useful in setting the time gate and for viewing each action potential.

Multiplexer

Fig A2.12

Components
- HEF 4052 Dual 4 channel anlog multiplexer
- 1/2 74LS13 Dual schmitt trigger
- 74LS10 Triple three input NAND
- 1/2 74LS73 Dual JK flip flop
- LM310 High speed voltage follower

The 74LS13 is wired as an oscillator whose output is passed into a 2 bit binary divider and used to determine which of the four inputs to the analog multiplexer are connected to the voltage

Fig. A7

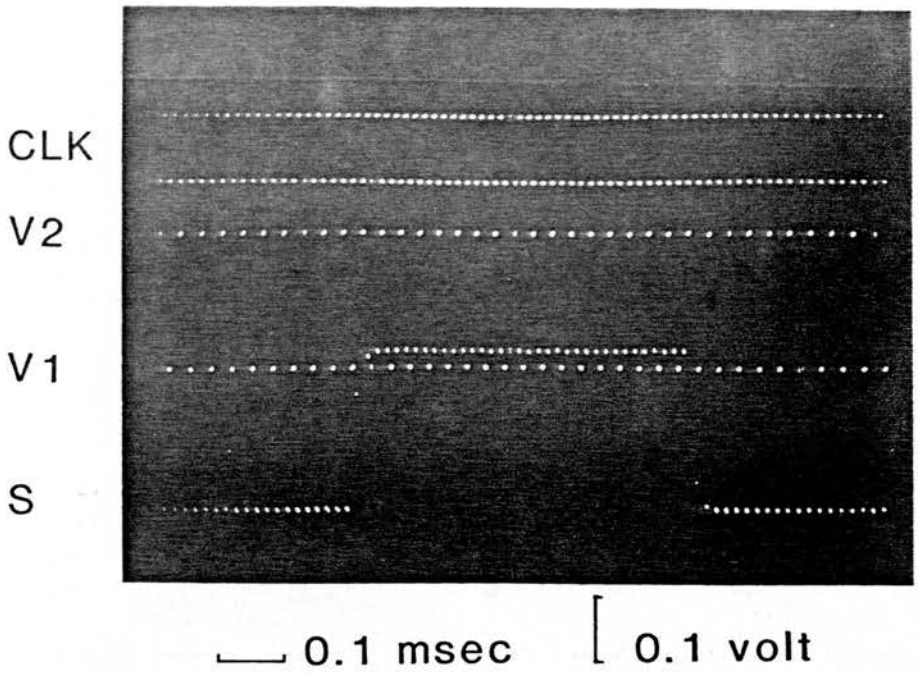


Fig. A8

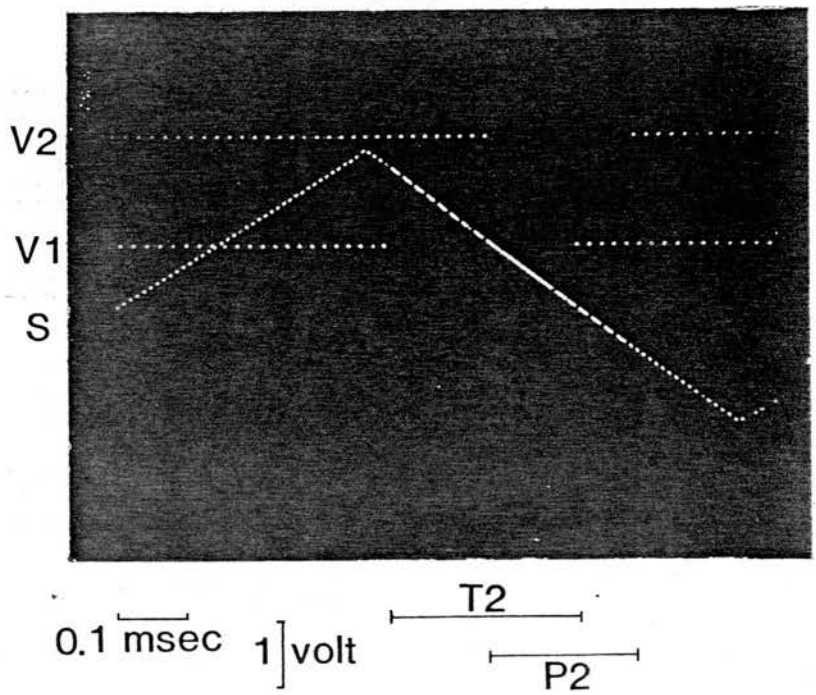


Fig A2.7-8 The Operation Of The Multiplexer

The multiplexer functions by sequentially switching V1,V2 and S into a single output. The clock, CLK, controls the switching.
In these two oscilloscope displays the time base is of sufficient speed to show switching between the three signals. At a slower speed the gaps in each signal are not apparent, Fig A2.2-6.

In Fig A2.7 gaps in V1 and V2 and hysteresis of V1 are not shown.

In Fig A2.8 the switching out of V1 in favour of S during T2 and of V2 during T2 is seen. This enables the operator to view the operation of the gate on a single channel oscilloscope. Hysteresis is also apparent.

follower output. The oscillator output pulses are not symmetrical and cannot therefore be used to produce switching periods of equal duration necessitating the use of a two bit divider. Input B of the multiplexer opens either inputs 1 or 3 to the output regardless of the state of A and as the nerve recording is connected to both it is displayed for 50% of the time in 2 1/4 periods. In addition the nerve recording is displayed during T2 at the expense of V1 and during P2 at the expense of V2, this is controlled by the 3 triple NAND gates. The switching in of the signal creates apparent gaps in the reference voltages increasing the information available to the operator. When the output of the multiplexer is displayed on an oscilloscope the following are displayed;

1) Nerve recording
2) Lower reference voltage
3) Upper reference voltage
4) Time Gate
5) Whether the signal has triggered an output pulse
6) Hysteresis on the lower reference voltage

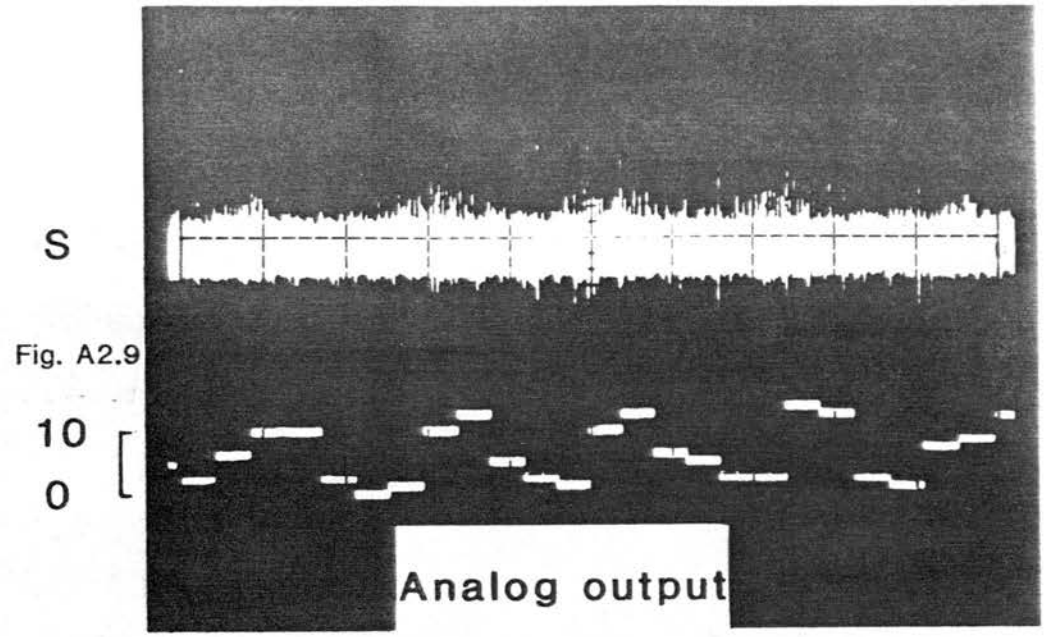
The multiplexer output is passed into LM310, a high speed voltage follower with a slew rate of 30 volts/microsecond, which buffers the output.

Analog counter

Fig A2.13

Components
74LS73 Dual JK flip flop
74LS74 Dual D type flip flop
2 x 74LS93 4 bit binary counter
2 x 74LS75 Quad D type flip flop
AD7520 10 bit digital/analog convertor
747 Dual 741 operational amplifier

The 74LS73 and 74LS93s form a ten bit binary counter into which output pulses from the gate are fed. At the end of each counting period the D type flip flops are clocked transferring the counts to the D/A convertor Which produces an analog voltage related to the



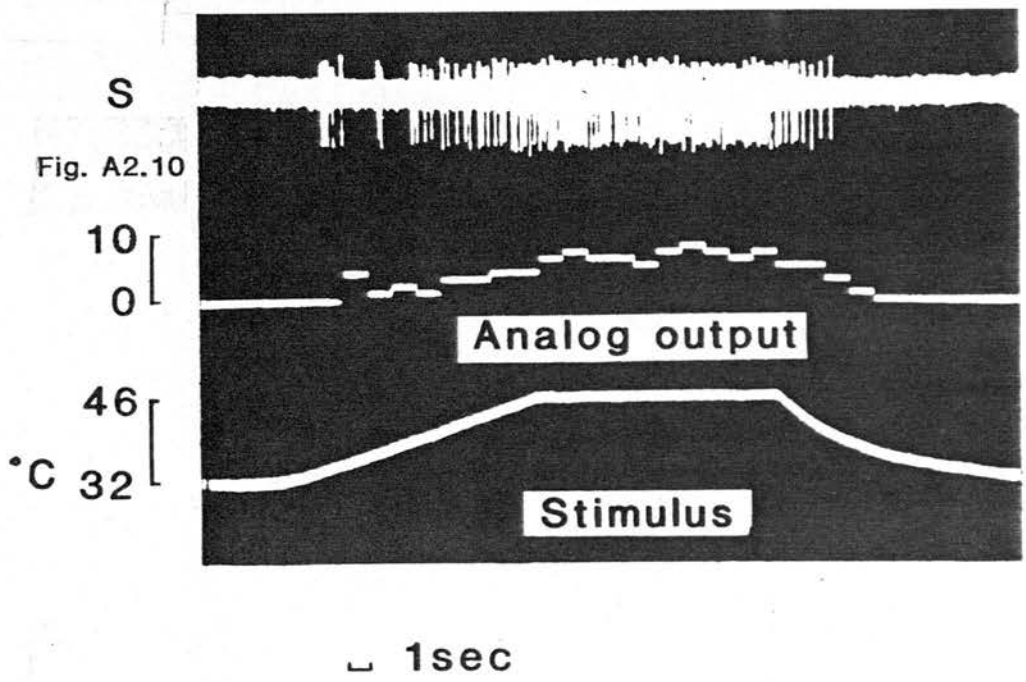


Fig A2.9-10 The Analog Output.

In Fig A2.9 the signal is of vagal afferents in the rat showing cardiac modulation in their discharge.

Fig A2.10 shows a thermosensitive unit in a chicken beak.
It responds to a temperature ramp and hold of 32-46°

digital input. The counting cycle is completed when the binary counter is cleared. The reference voltage for the D/A convertor is provided by a twelve volt zener diode and adjusted with a variable resistor which is used to set a 0-10v output range on the D/A convertor giving individual steps of approximately 10 millivolts.

Digital Counter

Fig A2.14

Components RS 587-024 Four digit multiplexed LED display
7217 CMOS Four decade counter driver

The 7217 counter driver contains all the components required to count over four decades, store the counts and drive a multiplexed display. Timing is provided by the timing section.

Timing

Fig A2.15

Components 4x 74LS90 Decade counter
74123 Dual Monostable
1/2 74LS13 Schmitt trigger
74LS00 Quad 2 input NAND

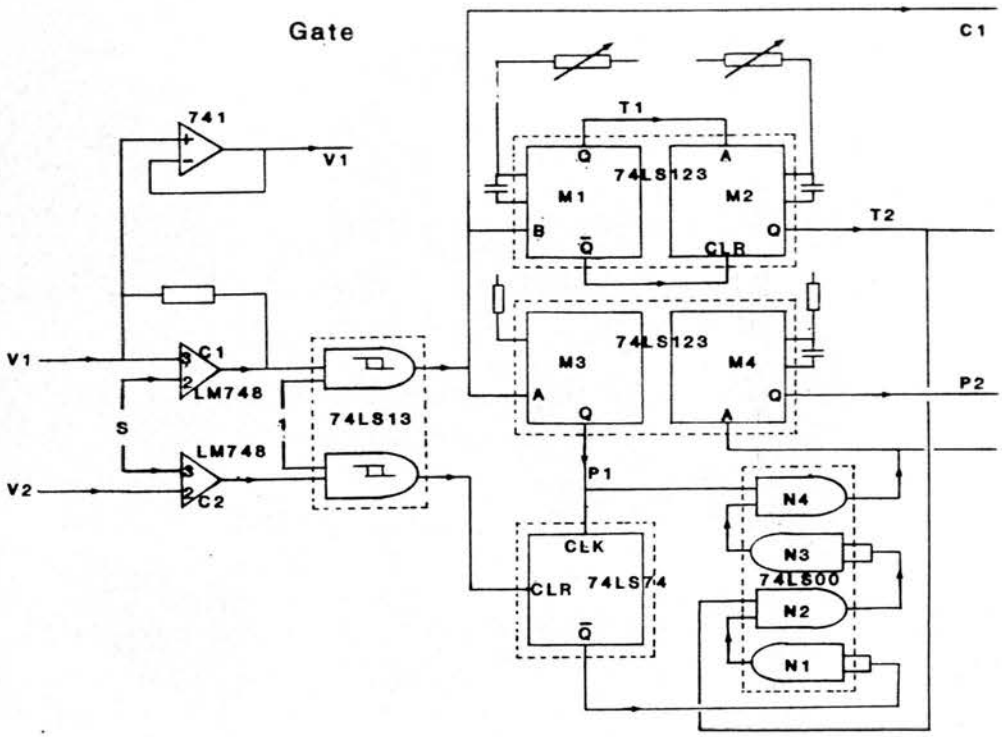
A 1000 Hz oscillator is built around the 74LS13 schmitt trigger and the output frequency adjusted using the 0-500 ohm variable resistor. The decade counters connected sequentially divide the output from the oscillator and switches S1 and S2 permit selection of the required counting period or test frequency.

Each cycle involves counting pulses, storing the counts on D type flip flops and clearing the counters. The output from S1 initiates the timing pulses, CK1 and CK2, which are generated by the sequential triggering of the 2 monostables. Storage of the counts occurs at the end of CK1 which is long enough, 60usec, to allow counting of pulses occuring prior to the start of CK1. CK2, 280usec, clears all counters. During the storage'clear interval the 74LS00 prevents pulses reaching the counters and causing an aberrant analog and digital output, the interuption of counting for a total of 340 usec in each cycle means that 1 pulse in 300 is lost with a counting period of 0.1 second.

The switch S2 selects freqencies in place of pulses derived form the gate and is used to calibrate the analog ouput.

An input through BC108 allows an external non TTL signal to determine the counting period.

Fig A2.11 Circuit Diagram Of The Gate.



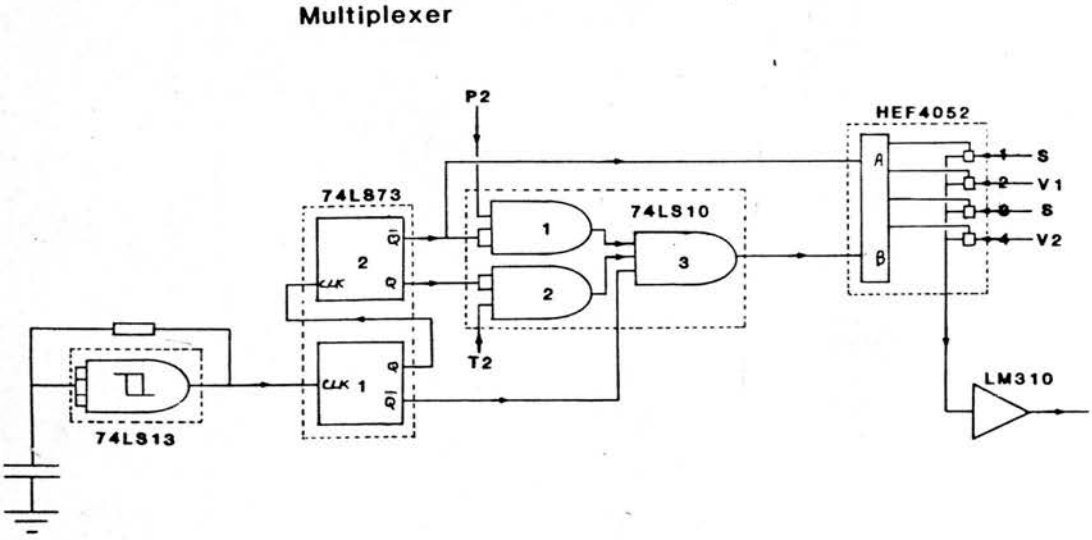


Fig A2.12 Circuit Diagram Of The Multiplexer

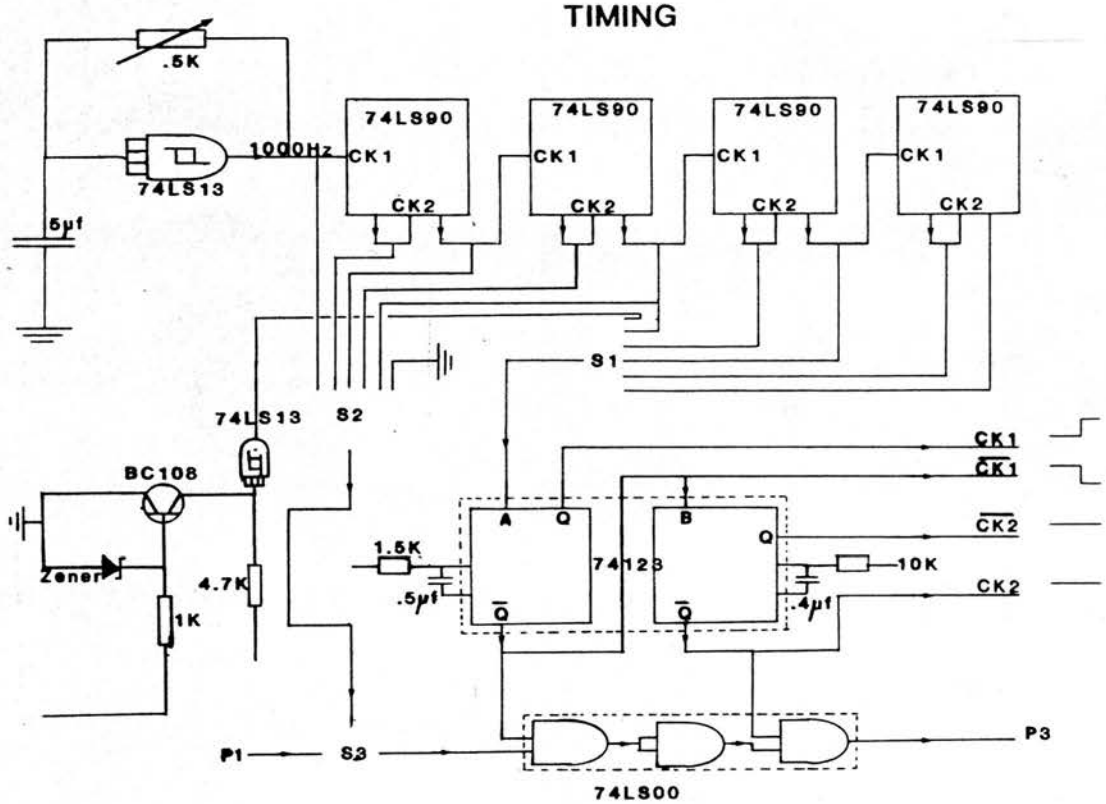


Fig A2.15 Circuit Diagram Of The Timing.

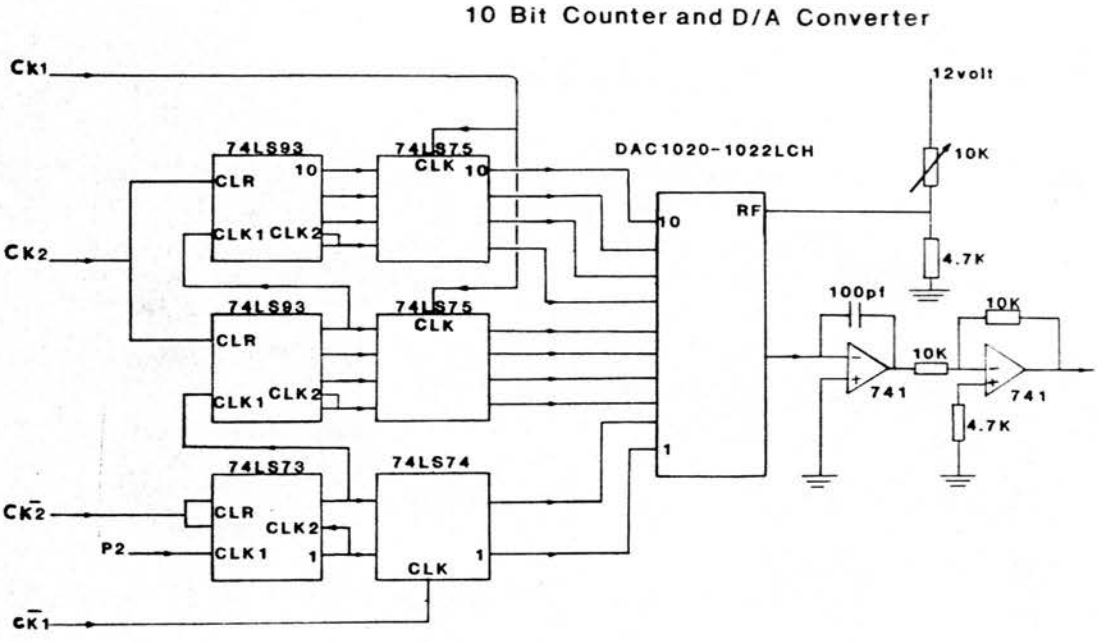


Fig A2.13 Circuit Diagram Of The Analog Counter.

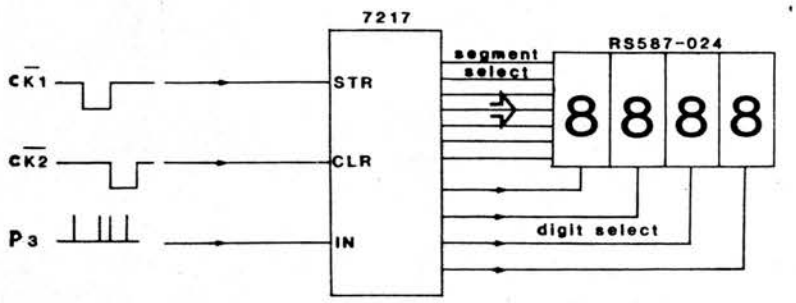


Fig A2.14 Circiut Diagram Of The Digital Counter.

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The cardiovascular actions of clonidine in the inactin-anaesthetized rat

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In the inactin (130 mg/kg I.P.), thiobutabarbitone, anaesthetized rat clonidine I.V. rapidly causes a prolonged bradycardia and hypotension after a brief hypertension. Pretreatment with atenolol (1 mg/kg) or atropine (1 mg/kg) show that heart rate is under sympathetic but not vagal control and that clonidine-induced bradycardia is only apparent in the presence of sympathetic tone.

Clonidine after atenolol pretreatment causes a fall in blood pressure without further bradycardia indicating an action on the vasculature. This is confirmed using a standard neurally intact hindlimb perfusion.

A modified 'delayed' hindlimb perfusion with a large extracorporeal circuit was used, blood born actions are delayed by the time taken to negotiate the extracorporeal circuit whilst neurally mediated actions are immediately apparent. Clonidine, Fig. 1, causes an immediate vasodilatation and a delayed vasoconstriction resulting in a net vasodilatation. The immediate neurally mediated vasodilatation outlasts the brief pressor response to I.V. clonidine and is therefore not solely a compensatory response. The delayed response to clonidine is pressor and no evidence for a peripheral vasodilator action on presynaptic alpha receptors is apparent, supporting Pichler & Kobinger (1978).

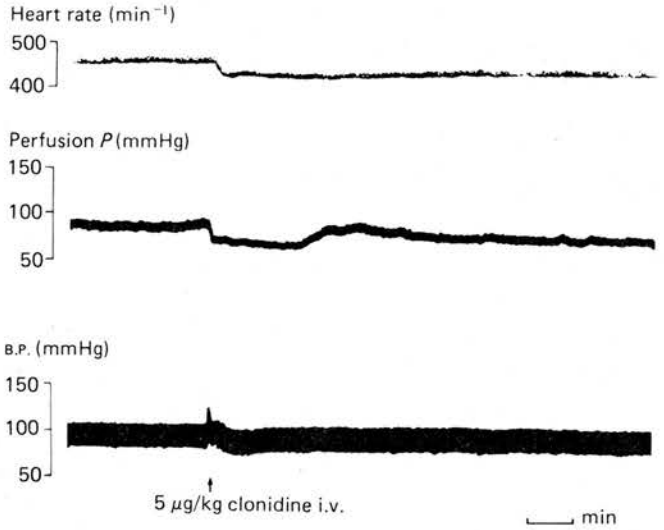

Fig. 1. The cardiovascular effects of I.V. clonidine on the 'delayed' hindlimb perfusion.

Schmitt, Schmitt, Boisser, Guidicelli & Fichelle (1968) showed that clonidine reduced sympathetic nerve activity and these results show a fall in peripheral resistance resulting from reduced nerve activity.

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An autoradiographic study into the site of action of clonidine in the rat

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In the anaesthetized rat clonidine causes a fall in blood pressure and bradycardia through a predominantly central action (Schmitt, Schmitt, Boissier, Guidicelli & Fichele, 1968). The site of action is not unequivocally established.

In these experiments clonidine was administered to 150–170 g inactin-anaesthetized rats (thiobutabarbitone 130 mg/kg I.P.) by a variety of routes and the hypotensive potency and resulting distribution of clonidine studied. Diffusible substance autoradiography with tritiated clonidine (Amersham) and the LKB tritium-sensitive film were used to locate the injected clonidine.

Intravertebral administration was achieved by retrograde injection into the right common carotid artery after ligation of all accessible branches of the right subclavian artery and, Fig. 1, was much more potent than intravenous dosing. Administration

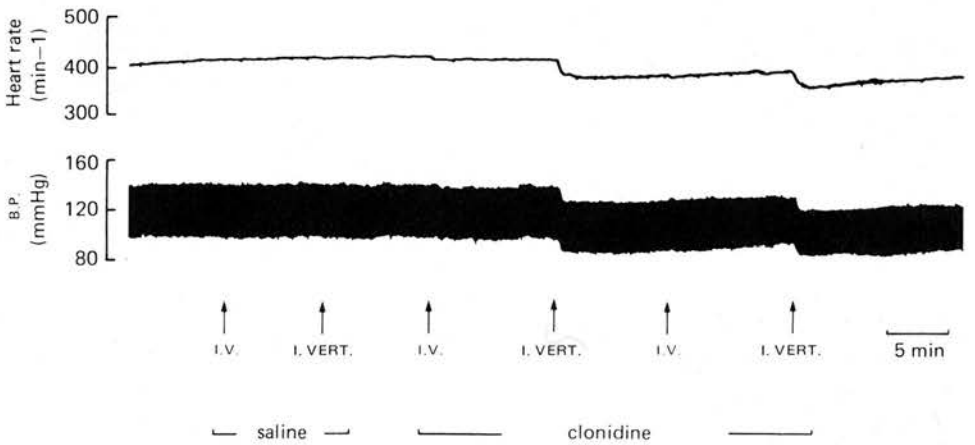

Fig. 1. Clonidine, 0·25 μg/kg, administration in an anaesthetized rat with an intravertebral, femoral venous and arterial cannulae. Injections in 40 μl. over 40 sec.

into the lateral ventricle, internal carotid artery or femoral vein were of similar potency although intraventricular injection resulted in a greatly reduced onset of action. The autoradiograms show that intravenously administered clonidine distributes evenly over the C.N.S. whilst other routes lead to high concentrations in different areas: intraventricular in areas bordering on the ventricular system and spinal canal, intracarotid in areas rostral to the medulla, intravertebral in the medulla, upper segments of the spinal cord, pons and caudal portions of the cerebellum. This suggests that in the rat the locus of clonidine's antihypertensive action is within the medulla or pons but not on the borders of the ventricular system.

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